Attorney Docket No. 9022-41 PATENT

In re: Maurer et al.
Application Serial No.: 10/767,352
Filed: January 30, 2004
Confirmation No.: 4884
Group Art Unit: 1618
Examiner: Blessing M. Fubara
For: *Oral Compositions of Fenretinide Having Increased Bioavailability and Methods of Using the Same*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

August 10, 2009

Commissioner for Patents
Post Office Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R § 1.132
OF BARRY J. MAURER, MD, PhD

Sir/Madam:

I, Barry J. Maurer, MD, PhD, do hereby declare and say as follows:

1. I received my PhD from California Institute of Technology. I received my medical degree from Wayne State University. I completed an internship at Children's Hospital of Michigan and a residency at LAC/USC Pediatric Pavillion in Los Angeles, CA. I also completed a clinical fellowship in pediatric oncology at Fred Hutchison Cancer Research Center and a research fellowship at Childrens Hospital Los Angeles Research Institute. I am further certified by the American Board of Pediatrics in hematology/oncology. I am currently an Associate Professor of Cell Biology, Pediatrics, and Internal Medicine at Texas Tech University Health Sciences Center in Lubbock, TX. A *curriculum vitae* is attached herewith at Appendix 1.

2. I am a co-inventor listed on U.S. Patent Application Serial No. 10/767,352 (hereinafter, "the '352 application"). I have reviewed the Office Action dated April 15,

2006 issued in association with the '352 application, and I am familiar with the contents thereof. I have also reviewed U.S. Patent No. 6,352,844 to Maurer et al. (of which I am a co-inventor); U.S. Patent No. 4,874,795 to Yesair; U.S. Patent No. 5,972,911 to Yesair; and U.S. Patent No. 4,665,098 to Gibbs, all of which are cited in the Office Action.

3. My efforts are dedicated to development of new drugs for use against childhood cancers. In particular, neuroblastoma, a type of cancer that affects the nervous system, typically occurs in children under 10 years of age. The previous (and largely ineffective) daily dosage of fenretinide, a synthetic vitamin A derivative, to treat this disease was 60 to 70 hard, oversized capsules. My colleagues and I knew we had to develop something better. Being pediatricians, we know that getting kids to take medicine is a challenge. We have now shown that fenretinide can not only be provided in a more palatable and more convenient way for patients, it can finally be absorbed into the blood at levels capable of shrinking tumors.

4. Regarding U.S. Patent Nos. 4,874,795 and 5,972,911 to Yesair, Dr. David Yesair invented the LYM-X-Sorb (LXS) lipid matrix to increase the oral absorption of water insoluble drugs. The LXS matrix composition that increased the oral absorption of fenretinide (4-HPR) while maintaining ease of manufacture was a 1:4:2 ratio of the constituent Lysophosphatidylcholine (LPC): Monoglycerides (MG): Free Fatty Acids (FA). *See* selected product information at Appendix 2. However, the 1:4:2 LXS matrix is a solid, bitter-tasting wax at room temperature (*See* figure at Appendix 3) rendering clinical patient compliance in the ingestion of fenretinide/LXS matrix wax quite difficult.

Cystic fibrosis patients generally have dysfunction of their pancreas, which leads to malabsorption of fats (lipids) and fat-soluble vitamins (A, D, E and K). The LXS wax (1:4:2 ratio) was pressed into a wafer and delivered to cystic fibrosis patients as a lipid nutritional supplement. *See* article at Appendix 4. However, the bitter taste of the LXS matrix decreased its clinical acceptance and medical compliance among the patients.

Accordingly, we worked with Dr. Yesair, and Avanti Polar Lipids, a manufacturer of LYM-X-Sorb, to create an oral powder composition containing fenretinide in the LXS wax matrix that would taste better, and also mix with liquids and foods, with the intent of increasing both the ease of administration and patient compliance. This was finally achieved by melting the LXS wax and mixing it with sugar and flour under closely controlled blending conditions. *See* photograph of fenretinide/LXS Oral Powder at Appendix 5. This composition was awarded the Eurand Award as Best New Oral Drug Formulation of 2004 by the international Controlled Release Society. *See* announcement at Appendix 6. Additionally, this new formulation was one of three semi-finalists and eventually won the Grand Prize at the 2004 Controlled Release Society Annual Meeting.

5. Regarding U.S. Patent No. 4,665,098 to Gibbs et al., Gibbs previously formulated fenretinide crystalline powder in a corn oil-containing capsule. This formulation was intended for delivering fenretinide for use as a chemoprevention drug in breast cancer. While the Gibbs composition increased fenretinide oral absorption compared to plain fenretinide crystalline powder alone, absorption was still poor. The daily intake of several capsules obtained only 1 – 3 micromolar blood plasma levels. Many capsules (up to 40 capsules per meter-squared of body surface area (BSA); adult = 1.7 m^2 BSA) were needed to obtain 7 – 10 micromolar plasma levels (*See* Garaventa, et al., "Phase I Trial and Pharmacokinetics of Fenretinide in Children with Neuroblastoma."Clin Cancer Res, 9: 2032-2039, (2003)). Ultimately, clinical responses using this formulation were limited (*See*, for example, Veronesi, et al., "Randomized Trial of Fenretinide to Prevent Second Breast Malignancy in Women With Early Breast Cancer." J Natl Cancer Inst, 91:1847-1856, (1999)), likely due to the low drug levels obtained in the blood, and the formulation was abandoned.

We tested fenretinide/LXS oral powder in mice. We found that fenretinide/LXS oral powder obtained much higher plasma and tissue levels than did the Gibbs formulation at equivalent doses. Further, the sugar-flour powder composition obtained higher drug levels in plasma and brain than did fenretinide in an LXS matrix that had not been so

powderized (for example, the non-powderized Yesair formulation). *See* Maurer, et al., "Improved oral delivery of N-(4-hydroxyphenyl)retinamide with a novel LYM-X-SORB organized lipid complex." Clin Cancer Res, 13(10):3079-3086, (2007), Figures 1, 2 and 3 comparing fenretinide/LXS oral powder to Gibbs/NCI capsule levels at 120 mg and 250 mg/kg/day or comparing fenretinide/LXS oral powder to fenretinide/LXS matrix at 120 mg/kg/day as shown at Appendix 7.

We then performed a Phase I clinical trial (a dose-tolerance finding study) in pediatric neuroblastoma in 32 patients (NANT 2004-04, NCI-funded, New Approaches to Neuroblastoma Therapy (NANT) Consortium, B. Maurer, Chair). We found that fenretinide/LXS oral powder had high patient compliance with taking the drug and obtained higher plasma drug levels than previously obtained using the Gibbs capsule formulation at equivalent doses. *See* data presented at Appendix 8. Further, above doses of 774 mg/m^2/day, 4 of 18 patients achieved Complete Responses in their tumors (no evidence of tumor), 2 patients of which are still tumor-free at +18 months. *See* Abstract titled "Phase I Study of fenretinide (4-HPR)/Lym-X-Sorb (LXS) oral powder in patients with recurrent or resistant neuroblastoma. A New Approaches to Neuroblastoma Therapy (NANT) consortium trial. Abstract #09-AB-31877-ASCOAM, Am Society Clin Oncology (2009) at Appendix 9. Previous studies with the Gibbs capsules produced only one such response in 54 patients. *See* Villablanca et al., "Phase I trial of oral fenretinide in children with high-risk solid tumors: a report from the Children's Oncology Group (CCG 09709)." J. Clin. Oncol. 24:3423-3430 (2006). These results demonstrate the clear superiority of fenretinide/LXS oral powder over previous oral fenretinide formulations, including the Gibbs formulation.

6. In summary, the formulation described in the '352 application provides a new formulation for the delivery of fenretinide that yields positive results including improved patient compliance, increased plasma concentrations and improved clinical success. The formulation described in the '352 application represents a new treatment for a childhood cancer that was difficult to treat.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Barry J. Maurer, MD, PhD

8/10/09
Date

APPENDIX 1

April 1, 2009

CURRICULUM VITAE

A. PERSONAL INFORMATION

Name in Full:	Barry James Maurer, M.D., Ph.D.
Business Address:	Texas Tech Health Sciences Center, STOP 6540, Room 5B109, 3601 4th St., Lubbock TX 79430
Business Phone:	(806) 743-2705
Home Address:	PO Box 66 Idalou, TX
Home Phone:	(806) 328-5298
Place of Birth:	Detroit, MI
Citizenship:	USA
E-Mail Address:	barry.maurer@ttuhsc.edu

B. EDUCATION

High School:	Notre Dame H.S., Harper Woods MI, 1976
College or University:	University of Michigan, Ann Arbor MI, B.S., B.S.E., 1980
Graduate School:	California Inst. of Technology, Pasadena CA, Ph.D., 1990 (Leave of Absence 1986 –1990)
Medical School:	Wayne State University, Detroit MI, M.D., 1990
Internship:	Children's Hospital of Michigan, Detroit MI, PGY 1, 1991
Residency:	LAC/USC Pediatric Pavilion, Los Angeles CA, PGY 2&3 Pediatrics, 1991-1993
Clinical Fellowship:	Pediatric Oncology Fellow, Fred Hutchinson Cancer Research Center, Seattle WA, 1993 -1997
Research Fellowship:	Childrens Hospital Los Angeles Research Institute, Los Angeles CA, 1997-2000

Honors and Awards:	1974-1978	National Merit Scholarship.
	1980-1986	NIH NRSA Predoctoral Traineeship.
	1987	Fellow, Summer Science Research Program for Medical. Students March of Dimes Birth Defects Foundation.
	1990	M.D. (Distinction in Biomedical Research).
	2004	Eurand Award Grand Prize, Novel Approaches in Oral Drug Delivery; Controlled Release Society, 31st Annual Meeting 2004: For novel LXS oral fenretinide formulation.
Licensure:	-California, Licensed Physician (G73799), 1992 - present -Washington, Licensed Physician (MD00030743), 1993-97	
Board Certification:	Am Board of Pediatrics, General Pediatrics (1993-2000). Am Board of Pediatrics, Hematology/Oncology (1998-2013).	

C. PROFESSIONAL BACKGROUND

ACADEMIC APPOINTMENTS:

2000 - 2007	Assistant Professor of Pediatrics and Cell & Neurobiology, Keck School of Medicine, The University of Southern California.
2007 - 2008	Associate Professor of Pediatrics, Keck School of Medicine, The University of Southern California.
2008 -	Associate Professor of Cell Biology, Pediatrics, and Internal Medicine, Texas Tech University Health Sciences Center, Lubbock , TX

CLINICAL APPOINTMENTS:

1998 – 2008	Attending Physician, Hematology/Oncology, Childrens Hospital of Los Angeles, Medical Staff Privileges.
1997 - present	Oncall Physician, Pediatric Hematology/Oncology, Medical Staff Privileges, City of Hope National Medical Center.

TEACHING RESPONSIBILITIES:

FORMAL INSTRUCTION/COURSES

- 1998 – 2008 CHLA Pediatric Resident Teaching Rounds, Ward Attending.
- 2001 – 2008 CHLA Heme/Oncology Fellow Educational Series, Lecturer. Core Topics: "Topics in Apoptosis"; "Strengths -Weaknesses of Animal Models in Cancer Developmental Therapeutics"
- 2001 – 2008 CHLA Summer Medical Student Program, Lecturer. Core Topics: "Developmental Therapeutics of Pediatric Cancer"
- 2001 – 2004, 2006 Heme/Oncology Journal Club, Presenter and Participant Core Topics: "Analyzing the Scientific Report - Examples"

ACADEMIC INSTRUCTIONAL PRESENTATIONS

- University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA; Cancer Center Grand Rounds – "Potential for Fenretinide-based Chemotherapy," 9/20/00.
- University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, Cancer Center Grand Rounds – "Clinical Potential of Ceramide Modulation: Laboratory and Translational Investigations." 09/23/03.
- CHLA Pediatric Grand Rounds – "Developmental Therapeutic Opportunities in Pediatrics" 3/25/05
- USC Cancer Center Grand Rounds, USC/Norris Comprehensive Cancer Center Seminar Series. "Progress update: sphingolipid modulation as chemotherapy." 2/14/06.
- USC-Caltech MD/PhD Seminar Series 9/25/06 "Developmental Therapeutics of Novel, Ceramide-Basaed Chemotherapies"
- Hematology Grand Rounds, University of Southern California/Norris Comprehensive Cancer Center, Los Angeles. "Phase I Trial of Fenretinide as Ceramide Modulator in Adult Leukemia/Lymphoma." 12/15/06.
- TTUHSC Department of Internal Medicine, Division of Biomedical Research Seminar. "Sphingolipid Modulation as a Novel Chemotherapeutic Approach to Anticancer Therapy," 11/25/08.

ACADEMIC MENTORSHIP

- 2000 – 2001 Medical Student Research Mentor
  Peter H. O'Donnell, MD, Howard Hughes Medical School Student Scholar Program. *Currently*: Clinical Fellow, Hematology/Oncology, Dept of Medicine, University of Chicago, 2006 – present.

- 2000 – 2003 Clinical Fellow Mentor
  Sandeep Batra, MD.
  Asst Prof., Hematology-Oncology, Childrens Memorial Hospital, Feinberg School of Medicine, Northwestern University, Chicago, IL 2003-2005. *Currently*: Staff, Avera St. Lukes Hospital, Aberdeen, SD 2005-present

- 2001 – 2003 Research Fellow Mentor
  Wei-Xing Guo, MD, PhD.
  Currently: Research Assistant Member, Free Radical Biology & Aging Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 2003-present

- 2003 – 2008 Research Scholar Mentor
  Hongtao Wang, MD, PhD.

- 2004 – 2007 Research Scholar Mentor
  Jun Wu, PhD.,
  Currently: Assistant Research Scientist
  Department of Clinical and Molecular Pharmacology
  City of Hope Beckman Research Institute
  Manager, Animal Tumor Model Core Facility
  City of Hope Comprehensive Cancer Center
  Duarte, CA 2007 - present

- 2005 - 2008 Graduate Student Supervisor/Thesis Advisor
  Yue "Alan" Zhang,

- 2005 – 2008 Dissertation Thesis Committee, Member
  Michael Hadjidaniel,

- 2005 Summer Oncology Medical Student Mentor
  Caroyln Girgis, B.S.

- 2006 – 2008 Clinical Fellow Research Guidance Committee, Member
  John Van Doorninck, MD.
  CHLA Heme/Onc Clinical Fellow, 2005 – present.

- 2007 – 2008 Clinical Fellow Research Guidance Committee, Member
  Tanja Gruber, MD. PhD.
  CHLA Heme/Onc Clinical Fellow, 2006 – 2008.

- 2008 - MD/PhD Graduate Student Supervisor/Thesis Advisor TTUHSC
  Michael Holliday

- 2008- MD/PhD Dissertation Thesis Committee, TTUHSC
  Jason Cooper

COMMUNITY SERVICE INSTRUCTION

- 2001 Wright Foundation Annual Meeting, University of Southern California, Los Angeles, CA; "Potential of Novel Retinoid Anticancer Drug Combinations," 5/21/01.

- 2001 – 2003 StopCancer Foundation, Los Angeles, CA Guest Speaker and Panelist, Invited Dinner Guest, "Fenretinide in Childhood Cancer,"

- 2001 – 2005 Neil Bogart/Martell Foundation, Fund-Raising Luncheon, Guest Speaker and Panelist, Laboratory Tour Proctor.

- 2001 – present Tyler's Team Leukemia Fund, San Marino High School, Annual Fund Raising Dinner, Guest Speaker, "Advances in ALL Leukemia"

- 2008 American Cancer Society – CBB Department Symposium on New Drug Development for Cancer, 11/18/2008.

- 2009 The Lubbock CEO Round Table – "The New TTUHSC Cancer Center and New Drug Testing" 01/17/09.

SPECIFIC ADMINISTRATIVE RESPONSIBILITIES (COMMITTEES)

2001 – 2008 Childrens Hospital Los Angeles Institutional Animal Care and Use Committee (IACUC), Member.

2001 – 2008 Childrens Hospital Los Angeles Intellectual Properties Committee (IPC), Member.

2001 – 2007 Childrens Hospital Los Angeles Summer Medical Student Program Reviewer, Lecturer, and Laboratory Research Supervisor.

2006 – 2008 USC, CHLA, and City of Hope

	Clinical and Translational Science Award (CTSA) Task Force Two Basic and Preclinical Working Group, Member.
2007 – 2008	CHLA Hematology/Oncology Fellow Research Guidance Committee
2008 -	TTUHSC Cell and Molecular Biology Graduate Program Committee

SPECIFIC ADMINISTRATIVE RESPONSIBILITIES (OTHER)

2004 – 2008	Supervisor, Animal Core, USC-CHLA Institute for Pediatric Clinical Research (IPCR).

PROFESSIONAL SERVICE

NATIONAL/CONSORTIUM COMMITTEE SERVICE

2001 – 2006	National Children's Oncology Group (COG) Second ALL Relapse Taskforce Lab Investigator - Biotherapy.
2002 – present	National Children's Oncology Group (COG) Member, Subcommittee, Neuroblastoma Developmental Therapeutics.
2002 – 2006	National Children's Oncology Group (COG) Member, Subcommittee, Developmental Therapeutics Protocol Development.
2005 – 2008	Therapeutic Advances in Childhood Leukemia (TACL) Consortium, Scientific Review Committee (SRC), Member.
2008 - present	National Children's Oncology Group (COG) Member, Subcommittee, Neuroblastoma Maintenance Therapy

INSTITUTIONAL COMMITTEE SERVICE

2001 – 2008	Childrens Hospital Los Angeles Institutional Animal Care and Use Committee (IACUC), Member.
2001 – 2008	Childrens Hospital Los Angeles Intellectual Properties Committee (IPC), Member.
2001 – 2008	Childrens Hospital Los Angeles Summer Medical Student Program Reviewer, Lecturer, and Laboratory Research Supervisor.
2004 – 2008	USC/Norris Cancer Center Leadership Council, Associate Member
2006 – 2008	USC Keck School of Medicine, Childrens Hospital Los Angeles, and City of Hope National Medical Center, NIH Clinical and Translational Science Award (CTSA) Task Force Two, Basic and Preclinical Working Group, Member.
2008 -	Member, TTUHSC Cell and Molecular Biology Graduate Program Committee.
2008 -	Member, TTUHSC Cancer Center Program Committee.

OTHER EMPLOYMENT OR INSTITUTIONAL ACTIVITY

2003 – 2004	USC/Norris Comprehensive Cancer Center, Associate Member
2004 – 2008	USC/Norris Comprehensive Cancer Center, Member
2004 – 2008	Member, USC/Norris Comprehensive Cancer Center Developmental Therapeutics and Clinical Trials Program
2004 – 2008	Member, USC-CHLA Institute for Pediatric Clinical Research
2008 -	Member, TTUHSC Department of Internal Medicine, Division of Biomedical Research

D. SOCIETY MEMBERSHIPS

- American Association for Cancer Research #73119
- International Society of Paediatric Oncology
- NCI-sponsored, National Children's Oncology Group (COG)

E. CONSULTANTSHIPS:

GRANT REVIEWS

2005	NIH/NCI P01 "Leukemogenesis Cluster" LKM-FLX grant cycle 1/2005, Adhoc Reviewer.
2006	Israel Science Foundation (ISF), Reviewer.
2007	NIH/NCI PO1 Program - Clinical Studies Special Emphasis Review Panel, grant cycle 1/2007, Reviewer. INVITED-excused for Conflict of Interest
2007	NIH NCI Oncological Sciences Integrated Review Group ZRG1 ONC-L (10) Cancer Drug Development and Therapeutics I SBIR/STTR Study Section, Reviewer.
2007 - present	NIH NCI Oncological Sciences Integrated Review Group Developmental Therapeutics (DT) Study Section, Reviewer.

2008 NIH Special Emphasis Panel, Special Topics in Biological Sciences, ZRG1 BCMB-B (90)
Ad hoc Reviewer.

2008 NIH NIGMS K99/R00 Special Emphasis Panel
ZGM1-BRT-9(KR), NIGMS-201570
Ad hoc Reviewer.

2008 SPARS AIBS FY2008 Department of Defense (DoD)
USAMRMC CDMRP PRMRP – Blood Cancer
Reviewer.

2001 - present REVIEWER FOR JOURNALS

- Journal of the National Cancer Institute
- Cancer Research
- Clinical Cancer Research
- Oncogene
- International Journal of Cancer
- Cancer Chemotherapy and Pharmacology
- Journal of Cellular Physiology
- Free Radical Biology & Medicine
- Molecular Cancer Therapeutics

F. RESEARCH ACTIVITES

MAJOR AREAS OF RESEARCH INTEREST

1. Molecular Pharmacodynamics and Developmental Therapeutics of Cytotoxic Retinoids and Modulators of Ceramide Metabolism as Novel Chemotherapy Therapeutic Approach in Pediatric and Adult Malignancies.

2. Molecular Characterization and Regulation of Dihydroceramide/Ceramide Cytotoxicity in Pediatric Neuroblastoma and ALL Leukemia Cell Lines.

3. Development of Pediatric ALL Leukemia Xenograft Models for New Agent Response and Pharmacokinetic/Pharmacodynamic Testing.

BIBLIOGRAPHY

PEER REVIEWED

1. McCombie, R.W., Hansen, J.B., Zylstra, G.J., **Maurer, B.J.,** and Olsen, R.H.: "Pseudomonas Streptomycin Resistance Transposon Associated With R-Plasmid Mobilization." *J Bacteriol* 165: 40-48 (1983).

2. **Maurer, B.J.**, Barker, P.E., Masters, J.N., Ruddle, F.H., and Attardi, G.: "Human Dihydrofolate Reductase Gene is Located in Chromosome 5 and is Unlinked to the Related Pseudogenes." *Proc Natl Acad Sci* USA 81:1484-1488 (1984).

3. **Maurer, B.J.**, Carlock, L., Wasmuth, J.J. and Attardi, G.: "Assignment of Human Dihydrofolate Reductase Gene to Band q23 of Chromosome 5 and a Related Pseudogene Ψ-HD1 to Chromosome 3." *Somat Cell Mol Genet* 11: 79-86 (1985).

4. **Maurer, B.J.**, Lai, E., Hamkalo, B., Hood, L.E. and Attardi, G.: "Novel Submicroscopic Elements Containing Amplified Genes in Human Cells." *Nature*, 327: 434-437 (1987).

5. Rasey, J.S., Cornwell, M.M., **Maurer, B.J.,** Boyles, D.S., Hofstand, P., Chin, L. and Cerveny, C.: "Growth and Radiation Responses of Cells Grown in Macroporous Gelatin Microcarriers (CultiSpher-G)." *Br J Cancer* 74 Suppl 27:78-81 (1996).

6. **Maurer, B.J.,** Ihnat, M.A., Morgan, C., Pullman, J., O'Brien, C., Johnson, S.W., Rasey, J.S., and Cornwell, M.M. "Growth of Human Tumor Cells in Macroporous Microcarriers Results in p53-independent, Decreased Cisplatin Sensitivity Relative to Monolayers." *Mol Pharmacol* 55:938-47 (1999).

7. **Maurer, B.J.**, Metelitsa, L.S., Seeger, R.C., Cabot, M.C., and Reynolds, C.P.: "N-(4-hydroxyphenyl)retinamide Increases Ceramide and Reactive OxygenSpecies and Induces Mixed Apoptoses/Necrosis in Neuroblastoma Cell Lines." *J Nat Cancer Inst* 91:1138-1146 (1999).

8. **Maurer, B.J.**, Melton, L., Billups, C., Cabot, M.C., and Reynolds, C.P. "Synergistic Cytotoxicity in Solid Tumor Cell Lines Between N-(4-hydroxyphenyl)retinamide and Modulators of Ceramide Metabolism." *J Natl Cancer Inst* 92:1897-1909 (2000).

9. Reynolds CP, Wang Y, Melton LJ, Einhorn PA, Slamon DJ, and **Maurer B.J.**: "Retinoic-acid resistant neuroblastoma cell lines show altered myc regulation and high sensitivity to fenretinide." *Med Ped Oncol* 35:597-602 (2000).

10. Wang, H., **Maurer, B.J.**, Reynolds, C.P. and Cabot, M.C. "N-(4-Hydroxyphenyl)retinamide Elevates Ceramide in Neuroblastoma Cell Lines by Coordinate Activation of Serine Palmitoyltransferase and Ceramide Synthase." *Cancer Res* 61:5102-5105 (2001).

11. *O'Donnell, P.H., *Guo, W-X., Reynolds, C.P., and **Maurer, B.J.** "N-(4-hydroxyphenyl)- retinamide Increases Ceramide and Is Cytotoxic to Acute Lymphoblastic Leukemia Cell Lines, but Not to Non-Malignant Lymphocytes." *Leukemia* 16:902-10 (2002).

12. Reynolds CP, Matthay KK, Villablanca JG, and **Maurer B.J.** "Retinoid therapy of high-risk neuroblastoma". *Cancer Lett.* 197:185-92 (2003).

13. Vratilova, J., Frgala, T., **Maurer, B.J.**, and Reynolds, C.P. "Liquid chromatography method for quantifying N-(4-hydroxyphenyl)retinamide and N-(4-methoxyphenyl)-retinamide in tissues." *Journal of Chromatography* 808:125-30 (2004).

14. Reynolds, CP, **Maurer B.J.**, and Kolesnik, RN: "Ceramide synthesis and metabolism as a target for cancer therapy." *Cancer Lett.* 206:169-180 (2004).

15. *Batra, S., Reynolds, C.P., and **Maurer, B.J.**, "Fenretinide Cytotoxicity for Ewing's Sarcoma (ES) and Primitive Neuroectodermal Tumor (PNET) Cell Lines is Decreased by Hypoxia and Synergistically Enhanced by Ceramide Modulators." *Cancer Res* 64:5415-5424 (2004).

16. Wu, X., Kim, Y., Sun, B-C., Moore, J.D., Shaw, W.A., and **Maurer, B.J.** "Liquid chromatography method for quantifying D-threo-1-Phenyl-2-palmitoylamino-3-morpholino-1-propanol (D-threo-PPMP) in mouse plasma and liver" *J Chromatogr B* 837(1-2):44-8 (2006).

17. **Maurer, B.J.**, Kalous, O., Yesair, D.W., Wu, X., Vratilova, J., Maldonado, V., Khankaldyyan, V., Frgala, T., Sun, B-C., McKee, R.T., Burgess, S.W., Shaw, W.A., and C. P. Reynolds, "Improved Oral Delivery of N-(4-hydroxyphenyl)retinamide with Novel LYM-X-SORB™ Organized Lipid Complex in Mice." *Clin Cancer Res.* 13:3079-3086 (2007).

18. Kong, G., Wang, D., *Wu, J., Konopleva, K., Andreeff, M., Ruvulo,P.P., and **Maurer, B.J.**, "Synthetic Triterpinoid Cytotoxicity in Pediatric Acute Lymphoblastic Leukemia Cell Lines is Independent of Ceramide Increase but Synergized by N-(4-hydroxyphenyl)retinamide." *Leukemia*, 22:1258-62, (2008).

19. Zhou J., Sohn, J., Spee, C., Ryan, S.J., **Maurer, B.J.**, Kannan, R., Hinton, D.R., "N-4-hydroxyphenyl retinamide augments laser-induced choroidal neovascularization in mice" *Invest Ophthalmol & Visual Sci*, 49:1210-20 (2008).

20. Wang, H.*, **Maurer, B.J.**, Liu, Y-Y., Wang, E., Allegood, J., Kelly, S., Symolon, H., Liu, Y., Merrill Jr., A., Gouaze-Andersson, V. , Yu, J., Giuliano, A., Cabot, M.C.: "N-(4-hydroxyphenyl)retinamide Increases Dihydroceramide and Synergizes with Dimethylsphingosine to Enhance Cancer Cell Killing." *Mol Cancer Ther* 7:2967-76. (2008).

21. Cheung E, Dorff T, Groshen S, Quinn DI, Reynolds, CP, **Maurer BJ**, Lara PN, Tsao-Wei DD, Twardowski P, Gandara DR, Chatta G, McNamara M, Pinski J: Oral fenretinide in biochemically recurrent prostate cancer: A California Cancer Consortium Phase II trial. *Clinical Genitourinary Cancer* 7:43-50 (2009).

22.

*denotes trainee.

NON PEER REVIEWED

1. Reynolds, CP, and **Maurer, B.J.**, "Evaluating Responses to Anti-Neoplastic Drug Combinations in Tissue Culture Models" *Methods Mol Med* 110:173-183 (2005).

2. Reynolds CP, Kang MH, Keshelava N, **Maurer B.J.**, "Assessing combinations of cytotoxic agents using leukemia cell lines." *Curr Drug Targets* 6:765-71 (2007).

ARTICLES IN PREPARATION

1. *Wang, H., Kong, G., Bielawski, J., and **Maurer, B.J.**, "Metabolism of safingol and its effects on endogenous sphingolipids formation in human neuroblastoma and ALL leukemia cell lines."

2. *Wang, H., Bielawski, J., Bielawska, A, and **Maurer, B.J.**, "Fenretinide-induced Sphingolipid Species are Differentially Modulated by Isomers of PPMP in Human Cancer Cell Lines".

3. Kalous, J., Maldonado, V., Sun, B-C., Chvilickova, I., Wu, X., Burgess, S., Shaw, W.A., Reynolds, C.P., and **Maurer, B.J.**, "D-threo-1-Phenyl-2-palmitoylamino-3- morpholino-1-

propanol (D-threo-PPMP) Increased Fenretinide-induced Ceramides and Xenograft Response in Human Neuroblastoma Models."

*denotes trainee.

ABSTRACTS

1. **Maurer, B.J.**, Barker, P.E., Masters, J.N., D'Eustacio, P., Ruddle, F.H. and Attardi, G.: "Chromosomal Location of the Normal Human DHFR Gene and Its Amplified Copies in Methotrexate-Resistant Cell Variants." (HMG7) *Cytogenetic Cell Genetics* 37: 534 (1984).

2. Lai, E., **Maurer B.**, Hamkalo, B.A., Hood, L. and Attardi, G. : "Identification of Novel Submicroscopic Extrachromosomal Elements Containing Amplified Genes in Human Cells by Pulse-field and Field-Inversion Gel Electrophoresis." *Leukemia* 1:276 (1987).

3. **Maurer B.J.**, Metelitsa L.S., Seeger R.C., Cabot M.C., and Reynolds C.P. "Fenretinide (4-HPR) Induces Ceramide in Neuroblastoma and 4-HPR Cytotoxicity is Increased by Modulators of Ceramide Metabolism." *Med Ped Oncol* 33:227, (1999).

4. **Maurer B.J.**, Cabot, M.C. and Reynolds, C.P. "Modulators of Ceramide Metabolism Synergize Fenretinide Cytotoxicity in Multiple Tumor Cell Types." *Proc Am Assoc Cancer Res* 41:239, (2000).

5. Reynolds CP, Villablanca JG, **Maurer BJ**, Chen RL, Stram D, Matthay KK, Seeger RC: "Biological therapy for minimal residual disease in neuroblastoma." The 10th Asian Congress of Pediatrics, Taipei, Taiwan, p. 10, (2000).

6. **Maurer B.J.**, Melton L, Billups C, Cabot MC, Reynolds CP: "Cytotoxicity of N-(4-hydroxyphenyl)retinamide (4-HPR) is increased by modulators of ceramide metabolism." *Med Ped Oncol* 35:742, (2000).

7. Wang, H., **Maurer, B.J.**, Reynolds, C.P., and Cabot, M.C. "N-(4-Hydroxyphenyl)retinamide Elevates Ceramide in Neuroblastoma Through Coordinate Activation of Serine Palmitoyltransferase and Ceramide Synthase." *Proc Am Assoc Cancer Res* 42: #1966 (2001).

8. O'Donnell, P.H., Reynolds, C.P., **Maurer, B.J.** "Clinically Achievable Levels of N-(4-Hydroxyphenyl)Retinamide Increase Ceramide and Are Cytotoxic for Human Acute Lymphoblastic Leukemia (ALL) Cell Lines." *Proc Am Assoc Cancer Res* 42: #1002 (2001).

9. Batra, S., **Maurer, B.J.**, Reynolds, C.P. "Fenretinide cytotoxicity in Ewing's sarcoma (ES) and primitive neuroectodermal (PNET) cell lines is blunted by hypoxia but synergistically enhanced by safingol." *Societe Internationale D'Oncologie Pediatrique* (SIOP) (2001).

10. Reynolds, C.P., O'Donnell, P.H., Batra, S. and **Maurer, B.J.** "Fenretinide (4-HPR) increases ceramide and is synergistically cytotoxic for tumor cells when combined with ceramide modulators." ***Joint International Congress on APL and Differentiation Therapy 2001*** Rome, Italy.

11. Guo, W-X., and **Maurer, B.J.** "Involvement of c-Jun N-terminal Kinase (JNK) Signaling Pathway in N-(4-hydroxyphenyl)retinamide - Induced Cytotoxicity in Neuroblastoma Cell Lines. *Proc Am Assoc Cancer Res* 43:Abst #2153, (2002).

12. Frgala, T., Vlckova, J., Sun, B-C., Gupta, S., Vishnuvajjala, B.R., Ernst, W., Fuji, G. **Maurer, B.J.**, and Reynolds, C.P. "Pharmacokinetics of oral and intravenous formulations of N-(4-hydroxyphenyl)-retinamide (4-HPR) in nude mouse xenograft models." *Proc Am Assoc Cancer Res* 43:Abst #1367, (2002).

13. **Maurer, B.J.,** Sun, B-C., Frgala, T., Vickova, J., Ernst, W.A., Fuji, G., Gupta, S., Vishnuvajjala, B.R., and Reynolds, C.P. "Fenretinide (4-HPR) and 4-HPR + safingol prolong survival in a xenograft model of human neuroblastoma." *Advances in Neuroblastoma Research* (2002).

14. Goto S, Shimada H, Sun B-C, **Maurer, B.J.**, Sohara Y, Scadeng M, Pollack H, Nelson, MD Jr, DeClerk YA, Reynolds CP: A xenograft model of human neuroblastoma bone metastases in immunodeficient (SCID) mice. *Advances in Neuroblastoma Research*, 2002.

15. Batra, S., **Maurer, B.J.,** Reynolds, C.P. "Fenretinide cytotoxicity in Ewing's sarcoma (ES) and primitive neuroectodermal (PNET) cell lines is blunted by hypoxia but synergistically enhanced by safingol." *Proc Am Assoc Cancer Res* 43: Abst #4584, (2002).

16. Goto, S., Shimada, H., Sun, B-C., **Maurer, B.J.,** Reynolds, C.P. "Defining the natural history of a metastatic human neuroblastoma model with PGP 9.5 immunocytochemistry." *Proc Am Assoc Cancer Res* 43: Abst #5022, (2002).

17. Wan, Z, Reynolds, C.P., **Maurer, B.J.** "Inhibition of glucosylceramide synthase and 1-O-acylceramide synthase by D,L-threo-PPMP synergized fenretinide cytotoxicity for neuroblastoma cells." *Proc Am Assoc Cancer Res.*44: Abst #2657, (2003).

18. Chen P, **Maurer, B.J.**, Yang B, Reynolds CP "Fenretinide-induced cytotoxicity, ceramide induction, and stimulation of ceramide synthetic enzyme activity in a neuroblastoma cell line are inhibited by hypoxia." *Proc Amer Assoc Cancer Res* 44: Abs #5552, (2003).

19. **Maurer, B.J.,** Reynolds, C.P., Yesair, D., Burgess, S.W., McKee, R.T. and Shaw, R. "High-Dose Fenretinide Delivery with Novel LYM-X-Sorb™ Organized Lipid Complex" *Liposome Advances, Prog in Drug & Vaccine Delivery,* 6th International Conference, London (2003).

20. Grigoryan, R., Keshelava, N., Sun, B-C., **Maurer, B.J.,** Ludeman, S.M., Colvin, O.M., and Reynolds, C.P., "Cyclophosphamide, but not melphalan or carboplatin, synergistically

enhanced topotecan activity against neuroblastoma cell lines in hypoxia." *Proc Amer Assoc Cancer Res* 45: Abs , (2004).

21. Wang, H., and **Maurer, B.J.** "Uptake and metabolism of safingol in human neuroblastoma and leukemia cell lines" *Proc Amer Assoc Cancer Res* 45: Abs #2980, (2004).

22. **Maurer, B.J.**, Reynolds, C.P., Yesair, D., Burgess, S.W., McKee, R.T. and Shaw, R "Improved oral fenretinide delivery with novel Lym-X-Sorb™ organized lipid complex" *Proc Amer Assoc Cancer Res* 45: Abs #7112, (2004).

22. Grigoryan, R., Keshelava, N., Sun, B-C., **Maurer, B.J.**, Ludeman, S.M., Colvin, O.M., and Reynolds, C.P., "Cyclophosphamide, but not melphalan or carboplatin, synergistically enhanced topotecan activity against neuroblastoma cell lines in hypoxia." *Advances in Neuroblastoma Research*, Genoa, Italy, 2004.

24. Gupta, S., Zgodinski, J., Vishnuvajjala, R., **Maurer, B.J.**, Reynolds, C.P., Solomon, D. "Product Development of Fenretinide, NSC 374551, Intravenous Emulsion Formulation." *Controlled Release Society*, 31st Annual Meeting, Hawaii (2004).

25. **Maurer, B.J.**, Reynolds, C.P., Yesair, D., Burgess, S., McKee, T., and Shaw, W., "High-Dose Oral Fenretinide Delivery with Novel Lym-X-SorbTM Organized Lipid Complex." *Controlled Release Society*, 31st Annual Meeting, Hawaii (2004). **NOTE: Abstract was awarded the 2004 CRS-Eurand Grand Prize Award for Novel Approaches in Oral Drug Delivery.**

26. Grigoryan, R., Keshelava, N., Sun, B-C., **Maurer, B.J.**, Sun, Bee-Chun, Ludeman, S.M., Colvin, O.M., and Reynolds, C.P., "Cyclophosphamide, but not melphalan or carboplatin, synergistically enhanced topotecan activity against Ewing's family tumor cell lines in hypoxia." Proc Amer Assoc Cancer Res., 46: Abs #4712, (2005).

27. Wang, H., and **Maurer, B.J.** "PPMP stereoisomers synergize fenretinide (4-HPR) cytotoxicity in human cancer cell lines in association with complex modulations of sphingolipid metabolism." Proc Amer Assoc Cancer Res., 46: Abs #4972 (2005).

28. Wan, Z., **Maurer, B.J.**, and Reynolds, C.P. "SiRNA for the LCBII, but not the LCBI Subunit of Serine Palmitoyl Transferase can suppress Fenretinide-stimulated de novo Ceramide Biosynthesis in Neuroblastoma Cell Lines." Proc Amer Assoc Cancer Res. 47:Abs #5506, (2006).

29. Kalous, O., Maldonado, V., Sun, B-C., Reynolds, C.P., and **Maurer, B.J.** "Fenretinide + D-threo-PPMP Prolongs Survival in Human Neuroblastoma Murine Xenograft Models." Proc Amer Assoc Cancer Res. 47:Abs #4339, (2006).

30. Wang, H. and **Maurer, B.J.** "Fenretinide Increased Ceramides Through *de novo* Synthesis and Inhibition of Sphingomyelin Synthesis in a Neuroblastoma Cell Line." Proc Amer Assoc Cancer Res., 47:Abs # 4653, (2006).

31. Wu, J., Wang, H., and **Maurer, B.J.** "Dihydroceramide desaturases are Differentially Expressed in Fenretinide-resistant Pediatric Acute Lymphoblastic Leukemia (ALL) Cell Lines." Proc Amer Assoc Cancer Res., 47:Abs #1257, (2006).

32. Wang, D., Kong, G., Wu, J., Ruvolo, P.P., Andreeff, M., Konopleva, M., and **Maurer, B.J.** "Synthetic Triterpenoid CDDO and its Derivatives Increase Ceramides and are Cytotoxic to Pediatric Acute Lymphoblastic Leukemia Cell Lines" Proc Amer Assoc Cancer Res., 47:Abs #4643, (2006).

33. Liu, X., Frgala, T.,Maldonado, V.,**Maurer, B.J.**, Reynolds, C.P. "Subcutaneous tumor exposure to fenretinide in neuroblastoma murine xenografts is below most tissues and potentially limits tumor responses." Advances in Neuroblastoma (ANR) meeting, Los Angles, May, 2006.

34. Keshelava, N., Kalous, K., Grigoryan, R., Anderson, C.P., **Maurer, B.J.**, Reynolds, C.P., "Formalized pre-clinical drug testing to facilitate clinical trial development in the New Approaches to Neuroblastoma Therapy (NANT) Consortium." Advances in Neuroblastoma (ANR) meeting, Los Angles, May, 2006.

35. Marachelian A, Janeb J, Villablanca JG, Maris JM, Reynolds CP, **Maurer BJ**. "Increased bioavailability of 4-HPR given as fenretinide/LYM-X-SORB® (LXS) oral powder in recurrent/resistant pediatric neuroblastoma: A New Approaches to Neuroblastoma Therapy (NANT) consortium trial." International Society of Paediatric Oncology (SIOP) 39th Annual Congress, Pediatric Blood and Cancer 49:477, 2007.

36. Liu X, **Maurer BJ**, Frgala T, Page JG, Noker PE, Fulton R, Ames MM, Reid JM, Gupta S, Vishnuvajjala R, Tomaszewsk JEi, Schweikar K, Reynolds CP. "Preclinical toxicology and pharmacokinetics of intravenous lipid emulsion fenretinide." AACR Molecular Targets and Cancer Therapeutics Meeting, 2007

37. Marachelian, A. Janeba, J. G. Villablanca, J. M. Maris, S. W. Burgess, W. A. Shaw, C. P. Reynolds, **B. J. Maurer.** "Fenretinide/Lym-X-Sorb (LXS) Oral Powder Increased Fenretinide Bioavailability in Recurrent Neuroblastoma." 3rd International Liposome Society Annual Meeting, London, 2007.

38. Mohrbacher A, Gutierrez M, Murgo AJ, Kummar S, Reynolds CP, **Maurer BJ**, Groshen S, Vergara L, Kang MH, Yang AS. "Phase I trial of fenretinide (4-HPR) intravenous emulsion for hematological malignancies." Amer Society of Hematology, *Blood*:110:2581, 2007.

39. Reynolds, C.P., Tran, N., Maldonado, V., Khankaldyyan, V., Shimada, H., **Maurer, B.J.**, "Fenretinide/Lym-X-Sorb oral powder combined with the oral microtubule inhibitor ABT-751 is highly active against multidrug-resistant neuroblastoma xenografts." #10031, Am Society of Clin Oncology Annual Meeting, 2008.

40. Cheung, E., Dorff, T., Groshen, S., Quinn, D.I., Reynolds, C.P., **Maurer, B.J.**, Twardowski, P., Gandara, D.R., Chatta, G., McNamara, M., Pinski, J. "Oral fenretinide in biochemically recurrent prostate cancer." #239, 2008 Genitourinary Cancers Symposium, Am Society of Clin Oncology Annual Meeting 2008.

BOOK REVIEWS

LETTERS TO EDITORS

CHAPTERS

1. Pauletti, G., **Maurer, B.J.**, and Attardi, G.: "Amplisomes: Novel Submicroscopic extrachromossomal Elements Containing Amplified Genes." In: *Gene Amplification in Mammalian Cells: Techniques and Applications.* Kellems, R.E. (Ed.) Dekker, New York (1991).

BOOKS

INTERNET PEER REVIEW

INTERNET NON PEER REVIEW

INTERNET PRESENTATIONS

INVITED LECTURES

- 2001 City of Hope National Medical Center, Duarte, CA;
  Pediatric Basic Medical Education Conference – "Potential of Fenretinide as Novel Anticancer Agent," 5/11/01.

- 2002 The Rebecca Guzner Memorial Pediatric Oncology Symposium, City of Hope National Medical Center, Duarte CA. "New Chemotherapeutic Targets and Agents in the Treatment of Neuroblastoma," 3/9/02.

- 2002 Cancer Affinity Group Seminar, The Scripps Research Institute, San Diego, CA. "The Generation and Modulation of Ceramide in vivo: Basis for a Novel Chemotherapy." 0 9/24/02

- 2002 M.D. Anderson Cancer Center, Houston TX;
  Pediatric Grand Rounds "Pediatric Applications of Fenretinide" 10/28/02.

- 2002 M.D. Anderson Cancer Center, Houston TX;
  Head and Neck Cancer Affinity Seminar "Fenretinide Applications in Adult Malignancies" 10/28/02.

- 2002 City of Hope National Medical Center, Duarte, CA;
  Pediatric Oncology Grand Rounds - " Fenretinide: Generation And Modulation Of Ceramide *In Vivo*.The Basis of a Novel Chemotherapy" 10/18/02.

- 2002 City of Hope National Medical Center, Duarte, CA;
  Pharmacology Seminar - " Fenretinide: Generation And Modulation Of Ceramide *In Vivo*. The Basis of a Novel Chemotherapy" 12/15/02.

- 2004 Southern California Biomedical Council 2004 Exposition - "Novel Ceramide-based Chemotherapeutic Agents." 03/24/04.

- 2005 University of California, San Francisco, Comprehensive Cancer Center Seminar Series – "Generation And Modulation Of Ceramide *In Vivo*: The Basis of a Novel Chemotherapy" 6/24/05

- 2006 2006 Gordon Conference on Glycolipid and Sphingolipid Biology, Ventura, CA, USA. "Generation and Modulation of Ceramides In vivo: the Basis of a Novel Chemotherapy." 1/10/06.

- 2006 The University of Texas M. D. Anderson Cancer Center's Apoptosis Working Group Seminar, "Ceramide Modulators in ALL Leukemia" 2/1/06.

- 2006 Advances in Neuroblastoma Research Conference, Los Angeles, CA – "Fenretinide + PPMP Prolonged Survival in Neuroblastoma Xenografts" 5/19/06.

- 2008 NIH NCI CTEP Early Drug Development Meeting (Fall)
  "Update on RAID-Supported Fenretinide/LXS Oral Powder and Fenretinide Intravenous Emulsion" 9/22-23/08.

- 2008 NIH NCI Clinical Therapeutics Evaluation Program (CTEP)
  "Development Session: Fenretinide Intravenous Emulsion" 9/29/08.

- 2009 NIH NCI CTEP Drug Development Group (DDG) Presentation
  "Clinical Development of Intravenous Fenretinide Emulsion," 1/05/09.

CLINICAL TRIALS/PROTOCOLS

NCI Individual Investigator Number: 35183

1. Phase I Study of Fenretinide (4-HPR, NSC 374551) Lym-X-Sorb®(LXS) Oral Powder in Patients with Recurrent or Resistant Neuroblastoma (IND #: 68,254). Chair: B.J. Maurer. Role: **Principal Investigator** STATUS: OPEN; NANT consortium; **FDA Investigational New Agent (IND) Sponsor - B. J. Maurer** 06/05.

2. A Phase I Study of Intravenous (Emulsion) Fenretinide (4-HPR, NSC 374551) in Children with Recurrent or Resistant Neuroblastoma (IND #70,058), Chair: B.J. Maurer. Role: **Principal Investigator** STATUS: OPEN (10/07); NANT consortium.

3. A Phase I Study of Intravenous (Emulsion) Fenretinide (4-HPR, NSC 374551) in Children with Recurrent or Resistant Acute Lymphoblastic Leukemia (ALL), Acute Myelogenous Leukemia (AML), and Non-Hodgkin's Lymphoma (NHL) (IND #70,058), PI: Theresa Harned. Role: **Vice-Chair, and Study Chair Mentor.** STATUS: OPEN (09/07); Therapeutic Approaches to Childhood Leukemia (TACL) consortium.

4. A Phase I Trial of Fenretinide/LYM-X-SORB® Oral Powder + ABT-751 in Children with Recurrent or Resistant Neuroblastoma. PI: A. Marachelian, MD. Role: Vice-Chair, FDA Investigational New Agent (IND) Sponsor, and Study Chair Mentor. STATUS: LOI-approved, NANT Consortium, 8/06.

5. A Phase II Trial of Fenretinide (4-HPR) in Biochemically Recurrent, Hormone Naïve Prostate Cancer. PI: J. Pinski. Role: Co-Investigator STATUS: Completed; USC/Norris (2003)

6. Phase I Trial of Intravenous Fenretinide Emulsion (4-HPR) for patients with Hematologic Malignancies. Local Protocol # PhI-42. PI: A. Yang. Role: Co-Investigator STATUS: OPEN; USC/UC Davis/City of Hope consortium; MD Anderson Cancer Center; NCI (2005)

7. A Phase I Study of IV Fenretinide in Patients with Malignant Solid Tumors. NCI Protocol #: 7540, Local #: OC-05-8/ PhI-54. PI: J. Pinski. Role: Co-Investigator STATUS: OPEN; USC/UC Davis/City of Hope consortium, (2007).

8. Phase I Study of Fenretinide (4-HPR, NSC 374551) Lym-X-Sorb®(LXS) Oral Powder in Adults With Solid Tumors and Lymphomas. NCI #P-07187. Chair: M.E. Gutierrez. Role: Co-Investigator STATUS: OPEN 10/07; NCI, Center for Cancer Research; **FDA Investigational New Agent (IND) Sponsor - B. J. Maurer** (2007).

NONCLINCIAL TRIALS/PROTOCOLS

1. Children's Oncology Group (COG) **ABTR04B1,** "Establishing Continuous Cell Lines and Xenografts from Pediatric Cancers for Biological and Pre-Clinical Therapeutic Studies." PI: C. Patrick Reynolds. Role: **Vice-Chair**. STATUS: OPEN (03/07).

RESEARCH GRANTS/AWARDS IN PAST FIVE YEARS

2000 StopCancer Foundation, Seed-Grant Award $15,000
Principal Investigator

2000 - 2006 Jeffrey Cusumano Leukemia Fund/ Hoefflin Foundation Awards $101,000
Principal Investigator

2001 - 2006 Tyler's Team Leukemia Fund Research Award $45,500
Principal Investigator

2001 – 2003 Chris Carrey Memorial/StopCancer Foundation Seed Grant Award $50,000
Co-Investigator

2000 – 2002 Wright Foundation Physician Scientist Award $100,000/yr x 2 years
Principal Investigator

2001 - 2004 StopCancer Foundation Career Development Award $50,000/yr x 3 years
Principal Investigator

2001 Children's Cancer Research Fund Research Grant $30,000
Principal Investigator

2001 American Cancer Society Institutional Research Grant $20,000
Principal Investigator

2001 - 2005 Neil Bogart/Martell Foundation $43,100/yr x 4 years
Principal Investigator
"Establishment of a NOD-SCID Xenograft model for *In Vivo* testing of new anti-leukemia drug combinations"

2002-2006 Whittier Foundation Translational Research Grant Reynolds (PI)
Co-Investigator "Intravenous Formulations of Fenretinide and the Ceramide Modulator Safingol" $176,00/yr x 3 years

2003 - 2004 **NIH NCI 1-R41-CA102842-01 Phase I FLAIR/STTR** Grant $249,969
Principal Investigator
"PPMP as a Ceramide Catabolism Inhibitor for Chemotherapy"

2003 - 2005 **NIH NCI 1-R21-CA102990-01** Reynolds (PI) $100,000/yr x 2 years
Co-Investigator,
"Xenograft models of human neuroblastoma bone metastases"

2003 - 2005 **NIH NCI 1-R43-CA92797-01A1 SBIR** Grant, E. (PI) (CHLA subcontract)
Grant Holder: Molecular Express, Inc. $81,840 x 2 years
Co-Investigator "Development of Liposomal Fenretinide and Safingol"

2004-2006 **NIH NCI Pediatric Preclinical Testing Program (PPTP)** Houghton (PI)
In vitro Screening Project – Reynolds (PI) Subcontract $8,000/yr x 2 years
Co-Investigator "New Agent Screening in Pediatric Cancers."

2005 -2008 USC-CHLA Institute for Pediatric Clinical Research (IPCR)
Princial Investigator, "New therapies for ALL leukemia" $257,000 x 3 years

ACTIVE GRANTS AS PRINCIPAL INVESTIGATOR

1. **NIH NCI 1-R01-CA100895-01A1** $165,000/yr x 4 years (NCE)
   Principal Investigator 07/01/04-5/30/09 25% Effort
   "Novel Ceramide-based Chemotherapy for Acute Leukemias"

2. **NIH NCI DTP Rapid Access to Intervention Development (RAID)** Grant $open-ended
   Principal Investigator, 05/08/03 - open 0% Salary Support
   "Formulation and IND-directed Toxicology of a Novel Oral Formulation of Fenretinide with Increased Ease of Administration and Increased Oral Bioavailability"

ACTIVE GRANTS AS CO-INVESTIGATOR

1. **NIH RO1 -GM077391** Cabot (PI) 10/07 – 09/12
   "Ceramide, Membrane Glycolipids and Glycoprotein Expression."
   Co-Investigator 09/08 – 8/10 5% Effort ~$58,000/yr x 2 years
   "Effect of GCS Inhibitor in Tumor Xenografts"

2. **NIH NCI CA 81403 PPG** Seeger (PI); Project 4
   Co-Investigator 04/01/05-3/31/10 10% Effort ~$16,000/yr x 5 years
   "Biology and Therapy of High Risk Neuroblastoma Program Project."
   These studies support the development of novel retinoid-based chemotherapies for neuroblastoma.

PATENTS AND INVENTIONS

1. **Maurer, B.J.**, Cabot, M. and Reynolds, C.P. *Treatment of Hyperproliferative Disorders.* **U.S. Patent #6,352,844; ISSUED March 5, 2002.**

2. **Maurer, B.J.**, Cabot, M. and Reynolds, C.P. *Treatment of Hyperproliferative Disorders.* **EPO #99930790.3; ISSUED April 11, 2008**

3. **Maurer, B.J.**, and Reynolds, C.P. *Treatment of Hyperproliferative Disorders.* **U.S. Patent #6,368,831; ISSUED April 9, 2002.**

4. **Maurer, B.J.**, Cabot, M. and Reynolds, C.P. Continuation-in-Part, *Treatment of Hyperproliferative Disorders.* P.C.T.A. No. PCT/US00/29996 (December, 1999) *(pending).*

5. Gupta, S., **Maurer, B.J.**, Reynolds, C.P., and Vishnuvajjala, B.R. *Pharmaceutical Composition of Fenretinide Having Increased Bioavailability and Methods of Using the Same.* **U.S. Patent #7,169,819; ISSUED January 31, 2007.**

6. Gupta, S., **Maurer, B.J.**, Reynolds, C.P., and Vishnuvajjala, B.R. *Pharmaceutical Composition of Fenretinide Having Increased Bioavailability and Methods of Using the Same.* **European Patent Office No. 1349545; ISSUED 12/13/08.**

7. Gupta, S., **Maurer, B.J.**, Reynolds, C.P., and Vishnuvajjala, R., *Pharmaceutical Compositions of Safingol and Methods of Use of the Same.* **European Patent Office No. 1594488; ISSUED** June 28, 2006.

8. Gupta, S., **Maurer, B.J.**, Reynolds, C.P., and Vishnuvajjala, R., *Pharmaceutical Compositions of Safingol and Methods of Use of the Same.* **U.S. Patent #7,476,692; ISSUED January, 13, 2009.**

9. **Maurer, B.J.**, Reynolds, C.P., Yesair, D.W., McKee, R.T., Burgess, S.W., and Shaw, W.A. Oral Compositions of *Fenretinide Having Increased Bioavailability andMethods of Using the Same. U.S. Patent Application No.10/767,352, Jan 30, 2004. Pub No.* US20050106216 A1, filed Jan 30, 2004 (pending); European Patent Office Application No.04706566.9 *(pending)*

10. **Maurer, B.J.**, Reynolds, C.P., Yesair, D.W., McKee, R.T., Burgess, S.W., and Shaw, W.A. *Oral Pharmaceutical Compositions and Methods of Using the Same.* U.S. Patent Application, No. 11/170,561, filed June 29, 2005 *(pending).*

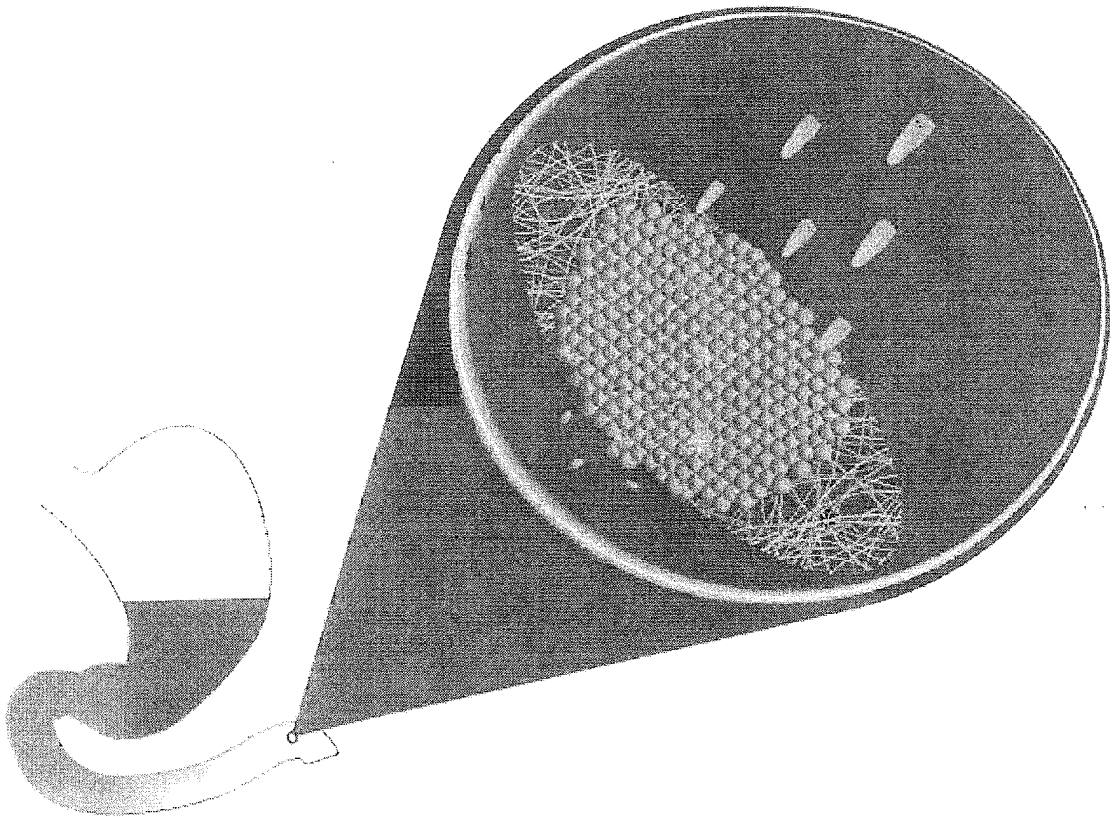
11. Reynolds, C.P., and **Maurer, B.J.**, *Drug Combinations for Treatment of Hyperproliferative Disorders.* USPTO Provisional Patent Application 60/800,954, filed May 17, 2006, PCT/US2007/011686, filed May, 2007

12. Reynolds, C.P. and **Maurer B.J.**, Metabolic Degradation Inhibitors for Anti-Hyperproliferative Agents. USPTO Provisional Patent Application filed Sept, 2007.

APPENDIX 2

A REVOLUTION IN ORAL DRUG DELIVERY:

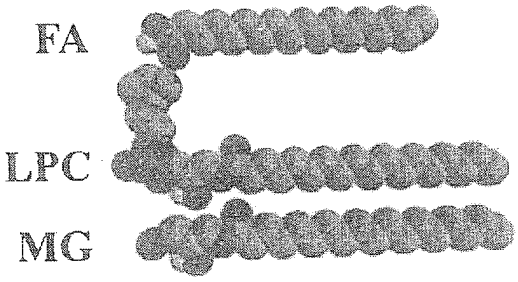
LYM-X-SORB™ (LXS™)

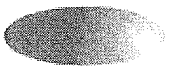


- Novel & Safe
- Maximum Drug Absorption
- Patent Protection

LYM-X-SORB™: Drug Compatibility

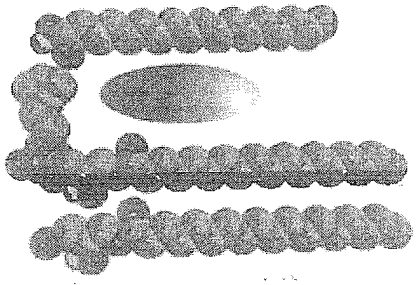
LYM-X-SORB™ Monomeric Structure





Drug

Improve Solubility and Stabilization of Drugs



LYM-X-SORB™/Drug Complex

Diverse Drug Structures

The following compounds have been shown to be compatible with the LYM-X-SORB™ matrix.

- Fenretinamide
- Insulin
- Histrelin
- ß Estradiol
- Nifedipin analogues
- Hydrochlorothiazide
- McN-5703
- Capsaicin
- Diltiazen
- Renin inhibitors
- Hydrocortisone
- Cromolyn
- Pramoxine
- Buprenorphine
- Progesterone
- Cyclosporin A
- Metronidazole
- Gentamicin

LYM-X-SORB™: Specifications

Composition

- LYM-X-SORB™ (LXS™) is composed of GRAS listed lipids accepted by FDA.
  - Lysophosphatidylcholine (LPC)
  - Monoglyceride (MG)
  - Free fatty acid (FA)
- Optimal range of molar ratios is 1:4:2 to 1:2:4, LPC:MG:FA.
- Molecular organization of LPC:MG:FA (1:4:2) as a monomeric structure is described on Page 2.

Physical Characteristics

- Eutectic organization demonstrates a single melting point which is lower than the melting points of the individual components.
- LXS™ adopts lamellar structural organization at approximately zero water content (See Page 5).
- LXS™ adopts purely hexagonal arrangement as water content approaches and exceeds 8 moles of water per mole of LXS™ (See Page 5).
- The LXS™ matrix can be a liquid or solid at room temperature by varying the unsaturation of the fatty acids.

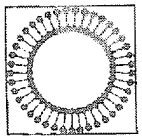
Aqueous Characteristics

- LXS™ has a low surface tension (25 dynes/cm).
- LXS™ is stable in physiological concentrations of sodium bicarbonate and bile salt (sodium taurocholate) present in the intestines.
- LXS™ forms small particles in physiological solutions (intestinal) [70nm to <10nm].
- Viscosity of LXS™ increases as the water content increases.

LYM-X-SORB™
History

BioMolecular Products, Inc.

David W. Yesair, Ph.D., former vice president of Arthur D. Little, Inc., founded BioMolecular Products, Inc., in 1984 to exploit lipid technology in both nutrition and drug delivery. He has invented, patented and licensed the LYM-X-SORB™ technology for such purposes. Dr. Yesair has formed LYM-MED Nutritional Products, LLC, for the purpose of delivering nutritionals to cystic fibrosis patients. This is now the subject of a license to an international pharmaceutical company.



Avanti® Polar Lipids, Inc.

Avanti Polar Lipids, Inc., founded in 1969 by Walter A. Shaw, Ph.D., has been manufacturing high purity lipids for over three decades. Avanti works closely with pharmaceutical and diagnostic partners to supply lipids meeting the stringent regulatory and purity requirements of a clinical product. Avanti manufactures lipids for numerous pharmaceutical and diagnostic products and currently supports more Drug Master Files than any other lipid supplier. Avanti's knowledgeable staff of chemists and biophysicists works with clients to provide information on formulation and lipid physical properties. Over the past 30 years, Avanti personnel have gained considerable insight and knowledge in the use of lipids as drug delivery vehicles.

LYM-DRUG PRODUCTS, LLC

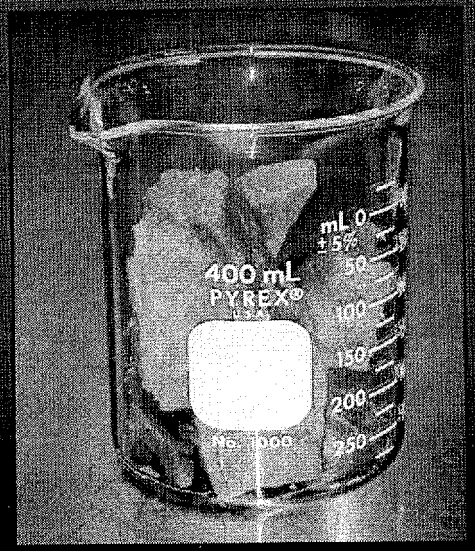
Dr. David W. Yesair of BioMolecular Products, Inc., and Dr. Walter A. Shaw of Avanti Polar Lipids, Inc., have developed a strong working relationship over the past two decades. They have formed LYM-DRUG PRODUCTS, LLC, in order to explore LYM-X-SORB™ as a revolution in oral drug delivery. This brochure provides an introduction to the LYM-X-SORB™ compatibility and enhanced oral bioavailability of drugs, its unique molecular and structural organization, its safety, and its proprietary position.

Contact us today to discuss how the LYM-X-SORB™ technology can improve your drug delivery.

Lym-X-Sorb™– Drug Formulation

Lym-X-Sorb Production

PC, MG, FA → PLA_2 (5-6 hrs) → LPC, MG, FA → vacuum dry (18-24 hrs) → +MG, +FFA → **LXS**



Drug Formulation
(0.8 Mole Ratio)

Powder Formulation
(25% LXS-Drug)

APPENDIX 4

Effect of an organized lipid matrix on lipid absorption and clinical outcomes in patients with cystic fibrosis

Guy Lepage, PhD, David W. Yesair, PhD, Nancy Ronco, RT, Josée Champagne, RT, Nathalie Bureau, BScN, Sylvain Chemtob, MD, PhD, Denis Bérubé, MD, and Claude C. Roy, MD

Objectives: To compare the absorption of a lysophosphatidylcholine, monoglyceride, and fatty acid matrix (organized lipid matrix, OLM) with that of a triacylglycerol (TG)-based fat meal in patients with cystic fibrosis (CF).

Study design: Five adolescents with CF and 3 control patients were given fat meals supplemented with retinyl palmitate of either OLM or TG at a 2-week interval. In a clinical trial, 73 patients with CF were randomly assigned to nutritional supplements containing either OLM or TG for a 1-year double-blind trial followed by a 6-month observation period.

Results: The peak increases and areas under the curve for TG and retinyl palmitate after the fat meal were 10-fold higher after OLM than after the TG fat load and did not differ from values obtained in control patients. OLM led to better clinical outcomes in terms of energy intake from the diet, weight-for-age *Z* score, essential fatty acid status, vitamin E, and retinol binding protein. Height-for-age *Z* score and FEV_1 only reached statistical significance at the end of the 6-month observation period.

Conclusions: These results suggest that OLM is a readily absorbable source of fat and energy in CF and is an effective nutritional supplement. (J Pediatr 2002;141:178-85)

Nutritional support is viewed as an integral part of the multidisciplinary cystic fibrosis (CF) care programs to prevent and correct growth failure and malnutrition known to be associated with an unfavorable prognosis.[1] A negative energy balance is central to the development of malnutrition in CF, and a low fat intake has been related to increased mortality rates.[2] However, it is clear that interventions must go beyond correction of the energy balance because CF is associated with specific nutrient deficits such as fat-soluble vitamins[3] and essential fatty acids (EFA).[4] The incidence of EFA deficiency in CF is high.[4] Although poorly correlated with nutritional status,[4,5] EFA deficiency has been associated with impaired growth[6] and hepatobiliary disease[7] as well as with increased vulnerability to lung infections.[8]

See editorial, p 157.

AUC	Area under the curve
ANOVA	Analysis of variance
BSA	Body surface area
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
EFA	Essential fatty acids
FA	Fatty acid
HAZ	Ideal height-for-age Z score
LPC	Lysophosphatidylcholine
MDA	Malondialdehyde
MG	Monoglycerides
OLM	Organized lipid matrix
PIVKA-II	Plasma prothrombin in vitamin K absence
PUFA	Polyunsaturated fatty acids
RBP	Retinol binding protein
RP	Retinyl palmitate
TG	Triacylglycerol
TX	Thromboxane
UDCA	Ursodeoxycholic acid
WAZ	Ideal weight-for-age Z score

The aim of the studies reported here was to explore the absorption after a fat meal of an organized lipid matrix (OLM) made up of lipolytic products and to investigate its efficacy as the lipid component of a nutritional supplement during a 1-year, double-blind clinical

From the Department of Pediatrics, Hôpital Ste-Justine, Université de Montréal, Québec, Canada.

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Reprint requests: Claude C. Roy, MD, Centre de Recherche, Hôpital Ste-Justine, 3175 Ch. Côte Ste-Catherine, Montréal, Québec H3T 1C5, Canada.

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trial followed by a 6-month observation period.

METHODS

Composition and Preparation of OLM

The OLM (LYM-X-SORB, BioMolecular Products, Inc, Byfield, Mass) is made up of the biliary and dietary products of lipolysis, namely, lysophosphatidylcholine (LPC), monoglycerides (MGs), and fatty acid (FA) in a 1:4:2 molar ratio. LPC was enzymatically derived from phosphatidylcholine (American Lecithin Co, Oxford, Conn) through the use of phospholipase A_2 (Novo Nordisk Bioch NA Inc, Franklinton, NC). MGs and FAs were purchased from Danisco Ingredients, Inc, New Century, Kan, and from Henkel Corporation Emory Group, Cincinnati, Ohio, respectively. The OLM preparation used for the fat load was prepared by BioMolecular Products, Inc; the one used for the clinical trial was the responsibility of Central Soya, Co, Inc, Fort Wayne, Indiana, but there was no difference between the two OLM preparations. The wafer cookies were confected by F and F Laboratories, Inc, Chicago, Ill.

In molar ratios of LPC/MG/FA varying from 1:4:2 to 1:2:4, the OLM yields a single melting point (64.6°C), which is lower than those of its individual components (88°C and 120°C for LPC, 69.6°C for MG, and 66.9°C for FA).[9] The molecular organization of this structured eutectic matrix is the result of the interaction of MGs with the acyl end of LPC while the negatively charged carboxyl group of the FAs interact with the negatively charged moiety of LPC. The critical micellar concentration of the OLM is 0.1 mmol, an order of magnitude less than the critical micellar concentration of bile salts.[9]

Absorption of a Liquid OLM Meal

Five adolescents with CF, 12 to 16 years of age, and 3 adult control patients were admitted to the Clinical Research Unit at Ste-Justine Hospital in Montreal. They were given a fat load of 29 g/m^2 of the OLM in the form of a sweetened and orange-flavored emulsion preceded, or followed 2 weeks later, by a conventional triacylglycerol (TG)-based nutritional supplement (Scandishake Scandipharm, Birmingham, Ala). The two isocaloric liquid meals given in conjunction with a retinyl palmitate (RP) capsule (48,000 IU/m^2, Roche, Montreal, Quebec, Canada) differed in terms of the fat, content, and composition. RP was added to measure the effect of the OLM on the absorption of lipid-soluble vitamins. The OLM liquid meal provided 854 µmol/g% of FAs versus 511 µmol/g% for Scandishake. A substantial proportion of fat energy in Scandishake was provided by medium-chain TG (15.1%) and monounsaturates (32.4%), whereas polyunsaturates were higher in OLM (55.8%) than in Scandishake (19.4%). No pancreatic enzyme supplements were taken with the two fat meals to test the hypothesis that the OLM does not need pancreatic enzymes to be absorbed and may contribute a good form of energy and fat in patients who respond poorly to pancreatic enzymes.[10]

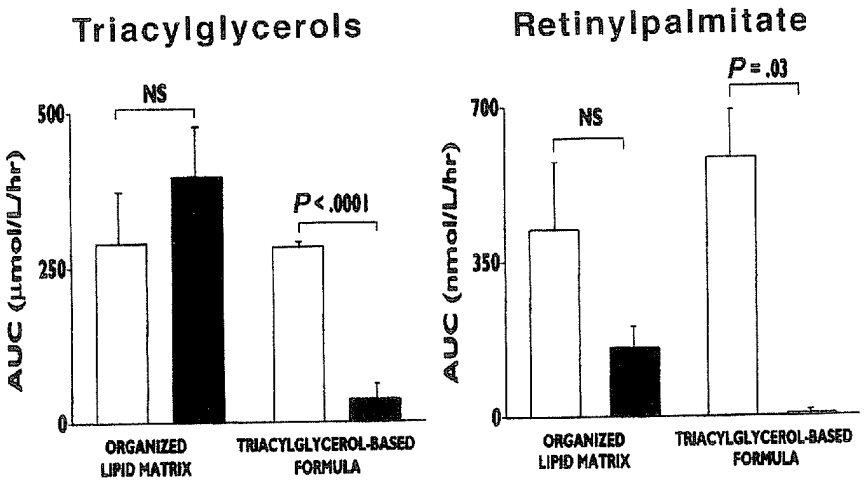


Fig 1. Absorption of the two liquid meals. Mean (± SEM) AUC for both TG and RP after OLM and TG-based formula fat load in 5 adolescents with CF *(black columns)* and 3 adult control patients *(white columns)*. Data were analyzed by ANOVA.

Blood samples were obtained from a small forearm-indwelling venous catheter at times 0, 2, 4, 6, 8, 10, and 12 hours. Plasma TG levels were determined by an enzymatic kit[11] and RP by high-performance liquid chromatography.[7] The peak plasma increases (Cmax) and the areas under the curve (AUC) over baseline values were plotted for TG and RP.

Clinical Trial With OLM

SUBJECTS. Over a period of 3 months, 78 children and adolescents with CF, 6 to 17 years of age (mean ± SEM, 11.7 ± 0.4 years) from the CF Clinic at Ste-Justine Hospital were invited to take part in a 1-year, randomized, double-blind, controlled trial with either OLM or TG as the lipid component of nutritional supplements. It was followed by a 6-month observation period to document whether any change in the EFA and in the clinical status could be sustained after termination of the trial. Seventy-three subjects agreed to participate (38 boys and 35 girls). Genotyping of this population revealed that 63.5% were homozygous and 25.8% were heterozygous for ΔF 508. All patients had biochemical evidence of EFA deficiency as evidenced by a plasma phospholipid 20:3n-9/20:4n-6 ratio of

Table I. Pretreatment characteristics of the two groups of patients who completed the study and of those who withdrew and who were noncompliant*

	OLM group (n = 22)	TG group (n = 26)	Patients lost (n = 25)
Boys/girls	9/13	12/14	17/8
Age (y)	11.1 ± 0.8	11.8 ± 0.6	12.1 ± 0.7
Height- for-age *Z* score	−0.66 ± 0.20	−0.85 ± 0.20	−0.70 ± 0.18
Weight-for-age *Z* score	−0.60 ± 0.16	−0.57 ± 0.19	−0.45 ± 0.19
Tricipital skinfold *Z* score	−0.32 ± 0.20	−0.59 ± 0.17	−0.40 ± 0.15
FEV_1 (% predicted)†	76.9 ± 2.6	84.7 ± 3.3	79.3 ± 4.1
18:2n-6(%)‡	17.9 ± 0.4	16.7 ± 0.5	17.2 ± 0.6
20:3n-9/20:4n-6 ‡	0.041 ± 0.004	0.037 ± 0.003	0.043 ± 0.005

There were no significant differences between the 3 groups for the variable shown (ANOVA).
*Mean ± SEM.
†Forced expiratory volume in 1 second.
‡Plasma phospholipid.

≥0.035 (≥4 SD over the control mean), a moderate degree of malnutrition, and/or growth failure (percent ideal weight for height) as well as decreased FEV_1 values (80.1 ± 2.4). Eight were receiving ursodeoxycholic acid (UDCA) (15 mg/kg per day) for hepatobiliary disease. Excluded from the trial were children with an FEV_1 <40 (percent predicted). Twenty children 5 to 17 years of age who had blood obtained for orthopedic conditions provided laboratory control values. Written informed consent was obtained from patients and/or from their parents after approval of the protocol by the Ethics Review Board of Ste-Justine Hospital.

TREATMENT PROTOCOL. The OLM and TG nutritional supplements were provided in the form of flavored isocaloric cookies (189 kJ or 45 kcal vs 202 kJ or 48 kcal/cookie, respectively) containing 5.2 g versus 5.8 g/cookie of carbohydrates, respectively, and 2.4 g of FAs. Polyunsaturated fatty acids (PUFAs) made up 50% of the FAs and consisted of a 5:1 mixture of n-6 and n-3. Packaged in air-tight wrappings, the cookies were kept at room temperature and were shown to be both physically and biochemically stable. The number to be ingested daily was calculated as a function of body surface area (BSA) (8 cookies/1.72 m^2 BSA). Patients were instructed to take their usual daily dose of pancreatic enzyme supplements with meals and the prescribed nutritional supplements. In view of the fact that an increased intake of PUFAs could enhance lipid peroxidation, all patients were asked to continue taking 400 units of α-tocopherol daily and to add 10,000 units of β-carotene daily (Pharmetics, Ltd, Montreal, Quebec, Canada).

Each patient was seen every 3 months by a CF clinic physician as well as by one of the two research nurses responsible for the trial. Weekly diaries kept by patients and their families were reviewed, and unused cookies were returned. Height and weight *Z* scores were recorded. At time 0, 6, 12, and 18 months, a 3-day diary of energy intake was recorded. At baseline, every 3 months during the 1 year trial, and twice during the 6-month observation period, blood counts, routine chemistries along with FA profiles,[4] vitamins A, E, and β-carotene,[12] and retinol-binding protein (RBP) were determined.[13] Vitamin D,[14] plasma prothrombin in vitamin K absence (PIVKA),[15] and eicosanoids PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane (TX) B_2[16] were obtained before and at the end of the trial. Pulmonary function testing was performed every 3 months, and the best of two reproducible FEV_1 values was recorded. Intravenous antibiotic days either at home or in the hospital were noted during the year before the trial as well as during and after the trial.

END POINTS AND SAMPLE SIZE. Our a priori hypothesis and the primary end point of the trial was that the OLM, as a more readily absorbable source of energy and PUFA than the usual TG-based nutritional supplements, would improve EFA deficiency. The secondary end points included improved nutritional status and growth as well as a reduced decline of FEV_1. We estimated that a sample size of 78 participants would be required to achieve 80% statistical power, with a 2-sided 5% level of statistical significance, assuming incomplete data were predicted to reach 30%. The analysis of the study was done as an efficacy trial comparing patients according to the treatment actually received and including only those who were compliant to the protocol.

STATISTICAL CONSIDERATIONS. Homogeneity of the treatment groups was ascertained for all baseline characteristics. All variables were summarized as mean ± SEM for continuous variables and frequency and proportions for categoric variables. Paired *t* tests were used to assess the significance of differences in one treatment group. To compare the absolute values and the mean relative percent change over time between groups, analysis of variance (ANOVA) was used.

For FEV_1 measurements, the slope (rate of decline) for each patient was estimated by linear regression analysis. This was done separately for the year before treatment, during the 12 months of treatment and the 6-month observation period. The means of the slopes obtained for each patient during these 3 periods were then analyzed by means of a 2-way mixed-model, repeated-measure ANOVA with groups and time as factors. Preliminary test on the group × time interaction was performed at a numerically high significance level (α = 0.25) to keep type 2 error (ie, accepting

the hypothesis of zero interaction when it should be rejected) relatively small, as suggested by Winer.[17] Then, under conditions in which group × time interaction was considered significant, the group slice effects were analyzed, and the differences between the groups were analyzed at each level of time.

All statistical analyses were performed with the use of SAS release 6.12-TS060. All tests were 2-sided, with an overall significance level of 5%.

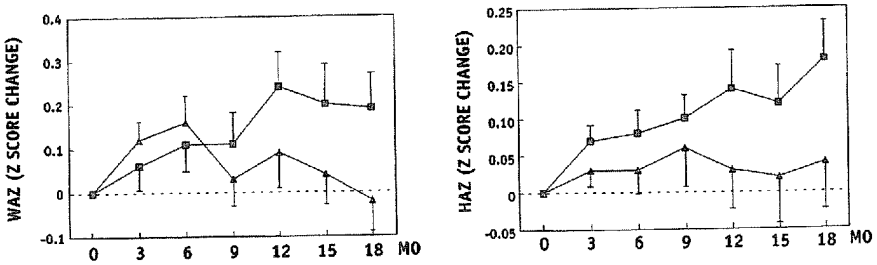


Fig 2. Change from baseline values of WAZ and HAZ during 1-year trial (3, 6, 9, and 12 months) and the 6-month observation period (15 and 18 months). Data for OLM (*black squares*, n = 22) and TG patients (*black triangles*, n = 26) were analyzed by 2-way (group × time) mixed-model growth curve analysis, in which time was considered continuous, to evaluate the slope of each group and to test their difference. For WAZ, the slope of the OLM was positive (0.01 ± 0.003, $P = .001$), whereas the slope of the TG group was negative (−0.008 ± 0.003, $P = .006$). Slopes for groups were different over time ($P = .0001$). For HAZ, the slope was positive (0.006 ± 0.002, $P = .0005$) for the OLM group, whereas the slope for the TG patients was not significant (0.00091 ± 0.002, $P = .62$). Slopes for groups were different over time ($P = .04$).

RESULTS

Absorption of the Two Liquid Meals

In response to the liquid meals, the AUC over baseline for the TG and RP in CF were 10-fold higher after OLM than after the TG-based formula. The increase of plasma TG and RP in response to the OLM in CF did not differ from control patients (Fig 1).

Clinical Trial

TRIAL PROFILE. Of the 73 patients randomly assigned to the two treatments, 6 patients withdrew (4 OLM and 2 TG) during the first week and 10 more later during the trial because of difficulties with compliance: 8 between 3 and 6 months (6 OLM and 2 TG) and 2 after 9 months (2 OLM). In accordance with the protocol and before breaking the randomization code, 9 subjects (4 OLM and 5 TG) who had completed the 1-year trial were removed for noncompliance to >75% of the prescribed cookies. The pretreatment characteristics of the patients lost did not differ from the 22 OLM and 26 TG who are the subject of the "per protocol analysis" (Table I).

ENERGY INTAKE. At baseline, the OLM subjects were consuming less energy from their diet than the TG group (114.2% ± 6.4% RDA *vs* 134.1% ± 7.0% RDA, $P < .05$). Using a 2-way (group × time), mixed-model, repeated-measures analysis of covariance controlling for baseline values to extract group simple effects, data from dietary records revealed that the intake of OLM supplements was associated with an increased intake of energy from meals at 12 months ($P = .004$). It was still manifest at the end of the 6-month observation period ($P = .03$). The percentage of prescribed cookies consumed did not differ between the two groups (86.2 ± 1.3 vs 89.6 ± 1.0), and their contribution to total energy intake was essentially the same (8.8% ± 0.4% vs 9.9% ± 0.5% RDA).

ANTHROPOMETRIC DATA. Because prepubertal growth spurt can render interpretation difficult, the distribution of patients in the 6- to 11.9-year and in the 12- to 18-year age categories was tested and found not to differ between the two arms of the trial ($P = .68$). Figure 2 shows in the OLM group a progressive increase in ideal weight-for-age Z score (WAZ), which was significantly different from the slope observed in the TG group both at 12 months and at the end of the observation period. With regard to ideal height-for-age Z score (HAZ), significance was only achieved at 18 months.

FATTY ACID PROFILES. No difference was observed in the total amount of phospholipid FAs among groups (Table II). Before initiation of the trial, 18:2n-6 and 22:6n-3 were significantly lower in both treatment groups than in control patients. Although concentrations of 20:4n-6 and 18:3n-3 did not differ from those in control patients, concentrations of 18:3n-6, 20:3n-6, 20:5n-3, and 22:5n-3 were statistically higher. Evidence of EFA deficiency was documented by the 20:3n-9/20:4n-6 ratio. It was 4 SD higher in both CF groups than in control patients and decreased significantly in response to treatment. Furthermore, there was an increase of 18:2n-6 coupled with decreases of both 20:4n-6 and 22:6n-3. These changes were more pronounced in the OLM subjects who, in contrast to the TG subjects, showed an increase of 18:3n-3. The plot of the change (%) over time of 18:2n-6 and 18:3n-3 concentrations calculated by the mixed-model repeated measures analysis of covariance controlling for baseline values revealed that the OLM was more effective than the TG supplements to improve both 18:2n-6 ($P < .02$) and the 18:3n-3 ($P < .003$) status (data not shown). Upon discontinuation of the supplements, a prompt return to baseline values was observed in both groups.

RBP, FAT-SOLUBLE VITAMINS, β-CAROTENE, AND MDA. The levels of RBP increased in both groups. In the children receiving OLM, the increase (41.9% ± 6.1%) was larger ($P = .01$) than in the

Table II. Fatty acid composition (mol%) of plasma phospholipids in control patients and in patients with CF before and after 1 year of treatment*

Fatty acids	Control group (n = 20)	OLM group (n = 22)		TG group (n = 26)	
		Baseline	After treatment	Baseline	After treatment
Total saturates	47.7 ± 0.4	46.6 ± 0.3	48.6 ± 0.3†a	46.8 ± 0.2	49.6 ± 0.3†b
Monounsaturates					
Total n-7	1.7 ± 0	2.1 ± 0.1‡c	1.9 ± 0.1	2.0 ± 0.1‡c	1.8 ± 0.1†b
Total n-9	11.7 ± 0.2	13.3 ± 0.25‡d	11.9 ± 0.3†a	13.4 ± 0.3‡d	11.1 ± 0.2†a
Polyunsaturates					
18:3n-3	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0
20:5n-3	0.6 ± 0	1.0 ± 0.1‡d	1.0 ± 0.1	1.0 ± 0	0.9 ± 0
22:5n-3	0.9 ± 0.1	1.1 ± 0	0.9 ± 0.1†b	1.1 ± 0.1‡e	1.0 ± 0.1
22:6n-3	2.7 ± 0.1	2.1 ± 0.1‡c	1.7 ± 0.1†a	2.1 ± 0.1‡c	2.0 ± 0.1†b
Total n-3	4.4 ± 0.2	4.5 ± 0.2	3.9 ± 0.1†a	4.4 ± 0.1	4.2 ± 0.1†b
18:2n-6	19.8 ± 0.5	17.9 ± 0.4‡d	19.7 ± 0.6†b	16.7 ± 0.5‡d	17.9 ± 0.5†b
18:3n-6	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.3 ± 0	0.1 ± 0
20:3n-6	2.8 ± 0.1	3.6 ± 0.1‡d	3.0 ± 0.1†a	3.7 ± 0.2‡d	3.4 ± 0.1†b
20:4n-6	9.3 ± 0.2	8.9 ± 0.2	7.9 ± 0.2†a	9.8 ± 0.3	9.3 ± 0.3†b
Total n-6	32.7 ± 0.4	31.4 ± 0.4	31.6 ± 0.6	31.4 ± 0.5	31.4 ± 0.4
20:3n-9/20:4n-6	0.015 ± 0.0001	0.041 ± 0.004‡d	0.031 ± 0.004†b	0.037 ± 0.003‡d	0.021 ± 0.002†a

*Mean ± SEM.
†Significantly different from baseline (paired *t* test): $^{a}P < .001$, $^{b}P < .01$.
‡Significantly different from control patients (Dunnett test): $^{c}P < .01$, $^{d}P < .001$, $^{e}P < .05$.

subjects receiving TG (25.2% ± 3.0%). With regard to retinol, 25-OH vitamin D, PIVKA-II, and β-carotene, no change was observed in response to treatment, and there was no difference between the two groups. In contrast, there was an increase ($P < .05$) of vitamin E in the children receiving OLM, whereas those receiving TG had no change. As expected, supplementation with EFA led to an increase of MDA during the trial, but there was no difference between the two groups over time (data not shown).

PULMONARY DISEASE. The slope of annual rate of change of FEV_1 (percent predicted) for each patient plotted from values obtained every 3 months during the year before the trial was not different in the OLM and the TG patients (Fig 3). In response to treatment, there was a trend toward a decrease of the annual rate of decline in the OLM compared with the TG group during the 12-month trial. Significance was achieved ($P < .02$) during the posttreatment observation period (Fig 3), as FEV_1 values in the patients with OLM improved, whereas there was a decline in the TG group. In the year preceding the trial, intravenous antibiotic days either at home or in the hospital of the OLM children (4.2 ± 1.7) did not differ from that of the TG group (3.5 ± 1.3). However, during the trial, there was a trend ($P = .11$) in the OLM group for fewer intravenous antibiotic days (2.3 ± 1.6) than in the TG group (8.2 ± 3.1).

LIVER BLOOD TESTS, LIPID PROFILES, AND EICOSANOIDS. Liver blood tests (AST, ALT, GGT), plasma TG, and cholesterol did not change in the 48 patients who completed the trial including those who were on ursodeoxycholic acid. Although there was no change in PGE_2, a fall of PGI_2 measured as 6-keto-$PGF_{1\alpha}$ was noted, and it was more pronounced in the OLM ($P < .001$) than in the TG patients ($P < .01$). TX_{A2} levels measured as TX_{B2} were significantly higher ($P < .001$) in both patient groups than in control patients at baseline. They fell significantly ($P < .05$) only in the OLM group (data not shown).

SIDE EFFECTS. There were no adverse effects directly attributable to the supplements. Monitoring of the amounts of pancreatic enzymes taken daily showed that there was no difference between the two groups. However, subjective reporting of digestive complaints revealed that several children receiving TG had a sensation of bloating and abdominal distension, whereas most children receiving OLM noted that they had a better appetite and that their stools were less malodorous, bulky, and greasy.

DISCUSSION

These studies show that a nutritional supplement with OLM as its lipid source has distinct advantages over one with TG in children and adolescents

with CF. In contrast to the very small increase of postprandial TG after a TG-based liquid meal, individuals with CF absorbed OLM as well as control patients. A 1-year trial with the OLM-based nutritional supplement produced better clinical outcomes than a comparable TG supplement in terms of nutritional status, growth, and pulmonary function.

Because OLM is made up of lipolytic products, it was expected to be well absorbed by patients with pancreatic insufficiency, especially since its LPC component, in contrast to phospholipids, is known to foster the absorption of lipids[18,19] and their delivery to lymph.[20] The observation that RP was better absorbed when given in conjunction with OLM than with the TG fat meal is of interest, since a higher incidence of vitamin E deficiency is reported in CF with a more severe degree of steatorrhea.[3]

It was legitimate to proceed with a *per* protocol analysis, comparing patients according to the treatment actually received and therefore including only those who followed the protocol properly, since it was a comparative trial. Because of the age variation of the subjects, progressive nature of the disease, the wide variation in illness severity, and the large intrasubject and intersubject variability, it was elected to express outcome measures over time as the change in response to the two treatment programs.

The contribution of the supplements to the total energy intake in both groups was modest (<10%) in comparison with reported nutrition supplementation programs, which commonly supply 20% to 30% of total energy.[21] The observation that throughout the trial as well as during the observation period, the OLM group consumed more energy from their meals than before the trial is intriguing and deserves to be investigated, especially since the TG group exhibited the decreased energy intake often reported when supplements are given.[10,21] Weight gain is a uniform finding and is commonly the only benefit of nutrition intervention program.[21] TG led only to short-term improvement of WAZ, whereas OLM was associated with a sustained and significant improvement in the rate of growth for both weight and height. The fact that significance for height was only reached at the end of the observation period is consistent with the lag period between weight and height observed in other studies.[22,23]

We have no data to support the possibility that OLM or one of its components such as LPC could be responsible for its impact on growth. One possible explanation for the LPC effect is that unlike dietary PL, PUFAs are not released from LPC upon digestion, but rather LPC may function as an acceptor system for the PUFAs of other dietary constituents. LPC is known to enhance FA absorption and delivery to lymph.[20] Although fat absorption was not measured during the clinical trial, it can be surmised that it was improved in the OLM group in view of the results of the acute fat load study.

The pattern of EFA deficiency in CF is unique and not a true deficiency because normal to increased levels of 18:3n-6, 20:3n-6, and 20:4n-6 are seen despite low levels of 18:2n-6.[24] This pattern, confirmed at baseline in this and in a previous study,[4] has been attributed to a higher rate of conversion of 18:2n-6 to 20:4n-6 acid in CF cells expressing the ΔF 508 cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.[25] In studies in which only 18:2n-6 is supplied, 20:4n-6 remains unchanged[26] or is even decreased.[21] In response to the supply of 18:2n-6 and 18:3n-3, both EFAs increased, but all of the FAs derived from their desaturation-chain elongation were decreased, including 20:4n-6 and 22:6n-3, and more so in the OLM group. Parent PUFAs have been noted to inhibit the synthesis of long-chain PUFAs.[27,28] As several authors have shown that increasing the energy intake in CF improves the EFA status,[29,30] it has been hypothesized that

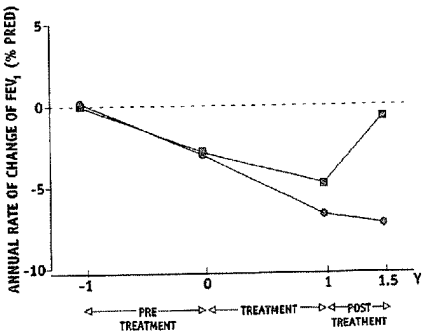


Fig 3. Effect of nutritional supplements on FEV_1 (as % predicted) estimated for each patient by means of simple linear regression and expressed as the annual rate of change. Data for OLM (*black squares*, n = 22) and TG patients (*black circles*, n = 26) were analyzed by means of 2-way (group × time) mixed-model repeated-measures ANOVA. Group × time interaction was significant at 0.25 level (P = .2128). Therefore, tests of group simple effects were performed. Significant difference was found after treatment (P = .02). Differences before treatment (P = .98) and during treatment (P = .67) were not statistically significant.

an energy deficit is the central problem in CF rather than malabsorption.[31] In this study, energy deficit is unlikely to be the explanation for the prompt return of the EFA status to its baseline values in both the OLM and TG groups, especially since the energy intake throughout the study was substantially greater in the former.

The fact that vitamin E levels were consistently higher in the OLM than in the TG group suggests that it was better absorbed, since we could not show a difference in compliance nor in the plasma levels of MDA. This is consistent with a previous study showing that the presence of LPC enhances the lymphatic absorption of α-tocopherol.[32]

The metabolism of prostanoids may be abnormal in CF. It is reported to be abnormal in patients with CF and the result of an abnormal turnover of arachidonic acid in membrane phospholipids.[33] The two treatments led to a decrease of PGI_2. However, TXA_2, known to play a role in bronchoconstriction and inflammation, decreased only in the OLM patients who had dur-

ing the study a 2-fold greater decrease of arachidonic acid than the TG group.

A primary goal of nutrition intervention programs in CF is not only to prevent or correct malnutrition and to improve growth but also to decrease the rate of progression of the pulmonary disease.[34-36] In the present study, the benefits of the OLM in terms of energy intake and growth continued beyond the period of nutrition intervention, and the favorable effect on pulmonary function only became evident during the posttreatment period. The trend in the OLM group for decreased pulmonary exacerbations might be related to the fact that better nutritional status is known to affect not only growth but also the long-term health of the lungs.[1]

In contrast to the TG-based fat meal and nutritional supplements, the OLM was as completely absorbed by patients with CF in the absence of pancreatic enzymes as by control patients and led to better clinical outcomes in terms of nutritional status, growth, and pulmonary function. These results showing that the OLM is a readily absorbable source of fat and energy in CF should be confirmed by a multicenter trial.

The authors would like to thank André Couturier and François Harel for their help in statistics, Mary Corey, Steve Cunnane, Larry Lands, and Elias Matouk for helpful discussions, Stan Shapiro and Louis Dumont for review of the manuscript, and Danielle St-Cyr Huot for expert secretarial assistance.

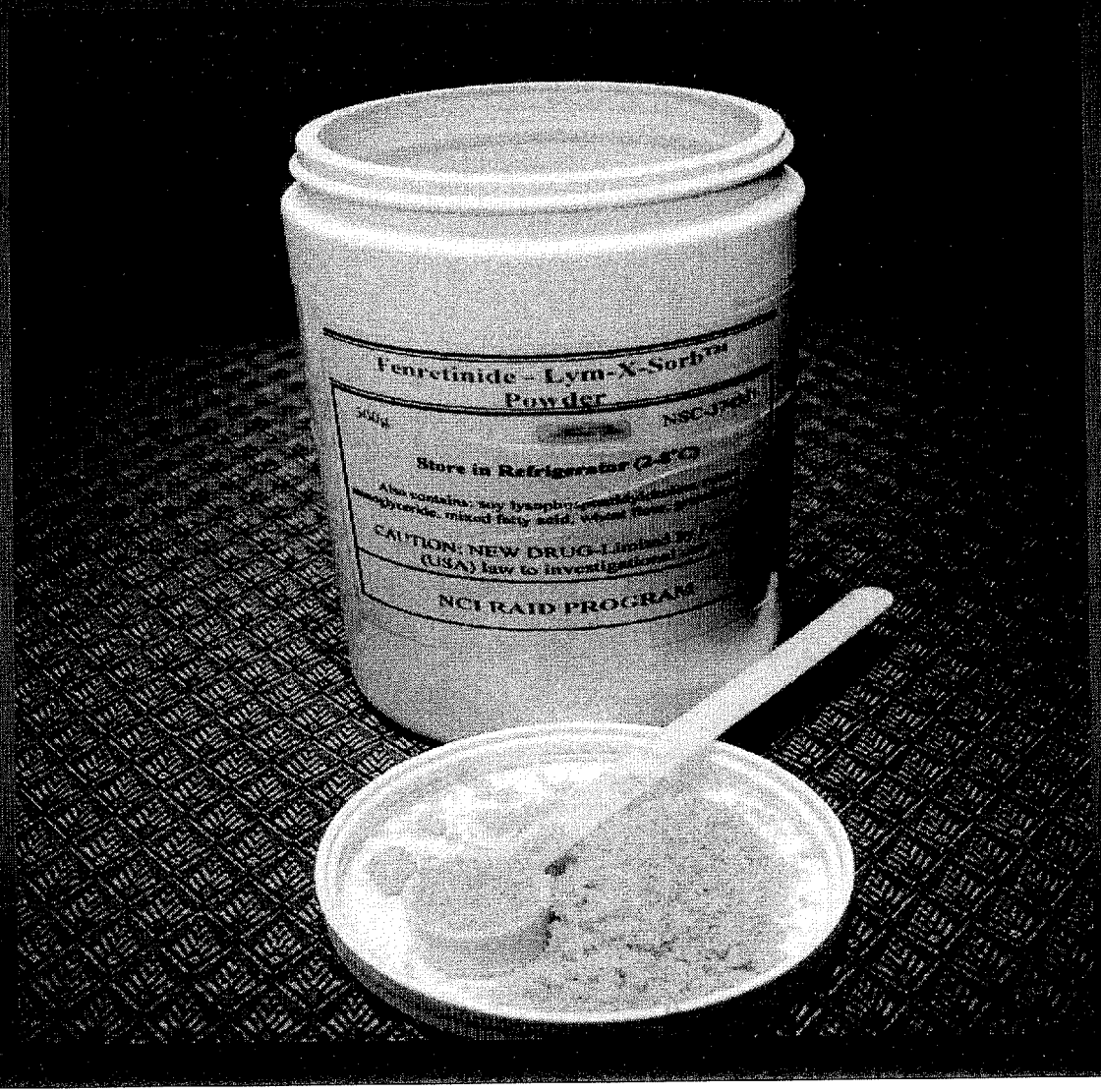
REFERENCES

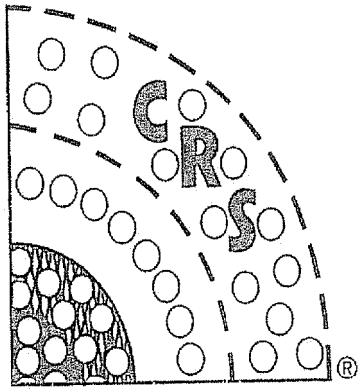
1. Ramsey BW, Farrell PM, Pencharz PB. Nutritional assessment and management in cystic fibrosis: a consensus report. Am J Clin Nutr 1992;55:108-16.
2. Corey M, McLaughlin FJ, Williams M, Levison H. A comparison of survival, growth, and pulmonary function in patients with cystic fibrosis in Boston and Toronto. J Clin Epidemiol 1988;41: 583-91.
3. Feranchak AP, Sontag MK, Wagener JS, Hammond KB, Accurso FJ, Sokol RJ. Prospective, long-term study of fat-soluble vitamin status in children with cystic fibrosis identified by newborn screen. J Pediatr 1999;135:601-10.
4. Lepage G, Levy E, Ronco N, Smith L, Galéano N, Roy CC. Direct transesterification of plasma fatty acids for the diagnosis of essential fatty acid deficiency in cystic fibrosis. J Lipid Res 1989;30: 1483-90.
5. Roulet M, Frascarolo P, Rappaz I, Pilet M. Essential fatty acid deficiency in well nourished young cystic fibrosis patients. Eur J Pediatr 1997;156:952-6.
6. van Egmond AWA, Kosorok MR, Koscik R, Laxova A, Farrell PM. Effect of linoleic acid intake on growth of infants with cystic fibrosis. Am J Clin Nutr 1996;63:746-52.
7. Lepage G, Paradis K, Lacaille F, Sénéchal L, Ronco N, Champagne J, et al. Ursodeoxycholic acid improves the hepatic metabolism of essential fatty acids and retinol in children with cystic fibrosis. J Pediatr 1997;130:52-8.
8. Lloyd-Still JD, Bibus DM, Powers CA, Johnson SB, Holman RT. Essential fatty acid deficiency and predisposition to lung disease in cystic fibrosis. Acta Paediatr 1996;85:1426-32.
9. Yesair DW. Phosphatidyl choline and lysophosphatidyl choline in mixed lipid micelles as novel drug delivery systems. In: Hanin I, Pepeu G, editors. Phospholipids. New York: Plenum Press; 1990. p. 83-106.
10. Pencharz PB, Durie PR. Pathogenesis of malnutrition in cystic fibrosis, and its treatment. Clin Nutr 2000;19:387-94.
11. Levy E, Lepage G, Bendayan M, Ronco N, Thibault L, Galéano N, et al. Relationship of decreased hepatic lipase activity and lipoprotein abnormalities to essential fatty acid deficiency in cystic fibrosis patients. J Lipid Res 1989;30: 1197-209.
12. Lepage G, Champagne J, Ronco N, Lamarre A, Osberg I, Sokol RJ, et al. Supplementation with carotenoids corrects increased lipid peroxidation in children with cystic fibrosis. Am J Clin Nutr 1996;64:87-93.
13. Benabdeslam H, Garcia I, Bellon G, Gilly R, Revol A. Biochemical assessment of the nutritional status of cystic fibrosis patients treated with pancreatic enzyme extracts. Am J Clin Nutr 1998; 67:912-8.
14. Grey V, Lands LC, Pall H, Drury D. Monitoring of 25-OH vitamin D levels in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2000;30:314-9.
15. Rashid M, Durie P, Andrew M, Kalnins D, Shin J, Corey M, et al. Prevalence of vitamin K deficiency in cystic fibrosis. Am J Clin Nutr 1999;70:378-82.
16. Varvarigou A, Bardin CL, Beharry K, Chemtob S, Papageorgiou A, Aranda JV. Early ibuprofen administration to prevent patent ductus arteriosus in premature newborn infants. JAMA 1996; 275:539-44.
17. Winer BJ. Design and analysis of factorial experiments. In: Winer BJ, editor. Statistical principles in experimental design. New York: McGraw-Hill Book Company; 1971. p. 309-430.
18. Rampone AJ, Long LR. The effect of phosphatidylcholine and lysophosphatidylcholine on the absorption and mucosal metabolism of oleic acid and cholesterol in vitro. Biochim Biophys Acta 1977;486:500-10.
19. Homan R, Hamelehle KL. Phospholipase A2 relieves phosphatidylcholine inhibition of micellar cholesterol absorption and transport by human intestinal cell line Caco-2. J Lipid Res 1998;39:1197-209.
20. Viola G, Mietto L, Secchi FE, Ping L, Bruni A. Absorption and distribution of arachidonate in rats receiving lysophospholipids by oral route. J Lipid Res 1993;34:1843-52.
21. Jelalian E, Stark LJ, Reynolds L, Seifer R. Nutrition intervention for weight gain in cystic fibrosis: a meta analysis. J Pediatr 1998;132:486-92.
22. Gaskin KJ, Waters DL, Baur LA, Soutter VL, Gruca MA. Nutritional status, growth and development in children undergoing intensive treatment for cystic fibrosis. Acta Paediatr Scand Suppl 1990;366:106-10.
23. Lai HC, Kosorok MR, Sondel SA, Chen ST, FitzSimmons SC, Green CG, et al. Growth status in children with cystic fibrosis based on the National Cystic Fibrosis Patient Registry data: evaluation of various criteria used to identify malnutrition. J Pediatr 1998; 132:478-85.
24. Christophe A, Robberecht E, Franckx H, De Baets F, Van de Pas M. Effect of administration of gamma-linolenic acid on the fatty acid composition of serum phospholipids and cholesteryl esters in patients with cystic fibrosis. Ann Nutr Metab 1994;38:40-7.
25. Bhura-Bandali FN, Suh M, Man SF, Clandinin MT. The Delta F508 mutation in the cystic fibrosis transmembrane

conductance regulator alters control of essential fatty acid utilization in epithelial cells. J Nutr 2000;130:2870-5.
26. Rettammel AL, Marcus MS, Farrell PM, Sondel SA, Koscik RE, Mischler EH. Oral supplementation with a high-fat, high-energy product improves nutritional status and alters serum lipids in patients with cystic fibrosis. J Am Diet Assoc 1995;95:454-9.
27. Manku MS, Morse-Fisher NM, Horrobin DF. Changes in human plasma essential fatty acid levels as a result of administration of linoleic acid and gamma-linolenic acid. Eur J Clin Nutr 1988;42:55-60.
28. Ackman RG, Cunnane SC. Long-chain polyunsaturated fatty acids. In: FB Padley, editor. Advances in applied lipid research. London: JAI Press; 1991. p. 161-215.
29. Mischler EH, Parrell SW, Farrell PM, Raynor WJ, Lemen RJ. Correction of linoleic acid deficiency in cystic fibrosis. Pediatr Res 1986;20:36-41.
30. Parsons HG, O'Loughlin EV, Forbes D, Cooper D, Gall DG. Supplemental calories improve essential fatty acid deficiency in cystic fibrosis patients. Pediatr Res 1988;24:353-6.
31. Hubbard VS. What is the association of essential fatty acid status with cystic fibrosis? Eur J Pediatr 1983;141:68-70.
32. Koo SI, Noh SK. Phosphatidylcholine inhibits and lysophosphatidylcholine enhances the lymphatic absorption of α-tocopherol in adult rats. J Nutr 2001;131:717-22.
33. Strandvik B, Svensson E, Seyberth HW. Prostanoid biosynthesis in patients with cystic fibrosis. Prostaglandins Leukot Essent Fatty Acids 1996;55:419-25.
34. Zemel BS, Kawchak DA, Cnaan A, Zhao HQ, Scanlin TF, Stallings VA. Prospective evaluation of resting energy expenditure, nutritional status, pulmonary function, and genotype in children with cystic fibrosis. Pediatr Res 1996;40:578-86.
35. Dalzell AM, Shepherd RW, Dean B, Cleghorn GJ, Holt TL, Francis PJ. Nutritional rehabilitation in cystic fibrosis: a 5 year follow-up study. J Pediatr Gastroenterol Nutr 1992;15:141-5.
36. Steinkamp G, von der Hardt H. Improvement of nutritional status and lung function after long-term nocturnal gastrostomy feedings in cystic fibrosis. J Pediatr 1994;124:244-9.

APPENDIX 5

Fenretinide - LXS™ Powder Formulation





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April 14, 2004

Dr. Walter Shaw
Avanti Polar Lipids, Inc.
U.S.A.
waltshaw@avantilipids.com

Dear Dr. Shaw:

CONGRATULATIONS! You have been selected to receive the 2004 **Novel Approaches in Oral Drug Delivery Award** sponsored by the Controlled Release Society and Eurand. The Eurand Award Committee has selected you as one of three recipients for 2004.

APPENDIX 7

Improved Oral Delivery of *N*-(4-Hydroxyphenyl)Retinamide with a Novel LYM-X-SORB Organized Lipid Complex

Barry J. Maurer,[1,2,3] Ondrej Kalous,[1,2] David W. Yesair,[4] Xiaqin Wu,[1] Jitka Janeba,[1] Vanessa Maldonado,[1] Vazgen Khankaldyyan,[1] Tomas Frgala,[2] Bee-Chun Sun,[2] R. Travis McKee,[5] Stephen W. Burgess,[5] Walter A. Shaw,[5] and C. Patrick Reynolds[1,2,3]

Abstract **Purpose:** Fenretinide [*N*-(4-hydroxyphenyl)retinamide (4-HPR)] is a cytotoxic retinoid that suffers from a wide interpatient variation in bioavailability when delivered orally in a corn oil capsule. The poor bioavailability of the capsule formulation may have limited responses in clinical trials, and the large capsules are not suitable for young children. To support the hypothesis that a novel organized lipid matrix, LYM-X-SORB, can increase the oral bioavailability of fenretinide, fenretinide in LYM-X-SORB matrix and in a powderized LYM-X-SORB formulation was delivered to mice.

Experimental Design: Fenretinide was delivered orally to mice as the contents of the corn oil capsule, in LYM-X-SORB matrix (4-HPR/LYM-X-SORB matrix) or in a LYM-X-SORB matrix powderized with sugar and flour (4-HPR/LYM-X-SORB oral powder). Levels of 4-HPR, and its principal metabolite, *N*-(4-methoxyphenyl)retinamide, were assayed in plasma and tissues.

Results: In a dose-responsive manner, from 120 to 360 mg/kg/d, delivery to mice of 4-HPR in LYM-X-SORB matrix, or as 4-HPR/LYM-X-SORB oral powder, increased 4-HPR plasma levels up to 4-fold ($P < 0.01$) and increased tissue levels up to 7-fold ($P < 0.01$) compared with similar doses of 4-HPR delivered using capsule contents. Metabolite [*N*-(4-methoxyphenyl)retinamide] levels mirrored 4-HPR levels. Two human neuroblastoma murine xenograft models showed increased survival ($P < 0.03$), when treated with 4-HPR/LYM-X-SORB oral powder, confirming the bioactivity of the formulation.

Conclusions: 4-HPR/LYM-X-SORB oral powder is a novel, oral drug delivery formulation, suitable for pediatric use, which warrants further development for the delivery of fenretinide in the treatment of cancer. A phase I clinical trial in pediatric neuroblastoma is in progress.

A synthetic retinoid made in the late 1960s, *N*-(4-hydroxyphenyl)retinamide (4-HPR; fenretinide), has been reported to be cytotoxic to, or inhibit the growth of, primary tumor cells, cell lines, and/or xenografts of various cancers, including those of neuroblastoma (1–3), colorectal (4), prostate (5, 6), breast (7, 8), ovarian (9–11), small-cell lung cancer (12), and both acute lymphoid and myeloid leukemias (13–15). Fenretinide cytotoxicity *in vitro* may be mediated through retinoic acid receptor–dependent and retinoic acid receptor–independent mechanisms (16), is p53 independent (12, 17–19), and can be caspase independent (19). Mechanisms of fenretinide cytotoxicity may involve reactive oxygen species generation (19–22) and/or an increase in ceramide species (saturated and desaturated *N*-acyl-sphingolipids; refs. 3, 15, 22–24).

Clinically, fenretinide has been studied in phase I, II, and III chemoprevention and chemotherapeutic trials using both low- and high-dose schedules using an oral gelatin capsule containing fenretinide (100 mg) in corn oil and polysorbate 80 [currently available through the National Cancer Institute (NCI)]. The systemic toxicity of fenretinide using chronic, low-dose schedules (generally 100–400 mg/d, obtaining ≤ 3 μmol/L plasma levels) has been minimal with the major clinical toxicity being reversible nyctalopia (decreased night vision) due to reduced plasma retinol levels (25). High-dose schedules (1,800 mg/m^2/d, divided into two or three daily doses, for 7 days, every 21 days) have also been well tolerated in adults (26). In pediatric cancer patients, occasional cases of hepatic toxicity and non-dose–related pseudotumor cerebri were observed in one pediatric phase I study, which determined a maximum tolerated dose at 2,450 mg/m^2/d (divided thrice daily, for 7 days, every 21 days; ref. 27); however, no dose-limiting toxicities were

Authors' Affiliations: [1]Developmental Therapeutics Program, USC-CHLA Institute for Pediatric Clinical Research and [2]Division of Hematology-Oncology, Childrens Hospital Los Angeles; [3]Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, California; [4]BioMolecular Products, Inc., Byfield, Massachusetts; and [5]Avanti Polar Lipids, Inc., Alabaster, Alabama

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Note: Certain intellectual property rights to fenretinide/LYM-X-SORB organized lipid matrix formulations may be reserved by Childrens Hospital Los Angeles (Los Angeles, CA) and/or BioMolecular Products, Inc. (Byfield, MA).

Requests for reprints: Barry J. Maurer, Childrens Hospital Los Angeles, Mail Stop 57, 4650 Sunset Boulevard, Los Angeles, CA 90027. Phone: 323-669-5663; Fax: 323-664-9455; E-mail: bmaurer@chla.usc.edu.

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observed in another pediatric study using the capsule formulation at up to 4,000 mg/m^2/d (single daily dosing, for 4 weeks, every 5 weeks; ref. 28). Dose escalation in this later study was terminated without reaching a maximum tolerated dose due to patient noncompliance with the number of capsules that needed to be consumed. However, even high-dose schedules of the capsule formulation have obtained relatively low micromolar plasma levels with a wide interpatient variation that has complicated interpretation of response data (26–28). Thus, developing improved formulations of fenretinide that obtain higher and/or more consistent plasma levels in a more patient-friendly dosing formulation could enhance 4-HPR antitumor activity.

LYM-X-SORB is an organized lipid matrix of lysophosphatidylcholine, monoglyceride, and free fatty acids, specifically designed to improve the solubility and oral bioavailability of drugs by forming chylomicron-like particles in the stomach and enhancing drug absorption via the lymphatics in the proximal intestine (29, 30). The LYM-X-SORB matrix is composed of U.S. Food and Drug Administration Generally Regarded As Safe list components and has a safety and tolerance showed in a 1-year double blind oral feeding study in cystic fibrosis patients (31). We have incorporated fenretinide into a LYM-X-SORB matrix (4-HPR/LYM-X-SORB matrix) and formulated it as a free-flowing powder, with roughly the flavor of raw cookie dough, for direct oral administration or for mixing in foods, juices, or nonmilk fat–containing liquid oral nutritional supplements. We report here that this new formulation, fenretinide/LYM-X-SORB oral powder (4-HPR/LYM-X-SORB oral powder), obtained significantly higher plasma and tissue levels in mice than did an equivalent dose of fenretinide delivered using the contents of the corn oil capsule. We also report that 4-HPR/LYM-X-SORB oral powder prolonged survival in two of three human neuroblastoma murine xenograft models. Additionally, it is anticipated that the powdered format of the formulation will be more acceptable to patients than the several dozen large fenretinide corn oil capsules currently required daily for an adult to obtain 4-HPR plasma levels >10 μmol/L. Thus, 4-HPR/LYM-X-SORB has the potential to improve the clinical anticancer activity of fenretinide by increasing drug systemic exposure, decreasing interpatient variability in absorption, and increasing patient compliance.

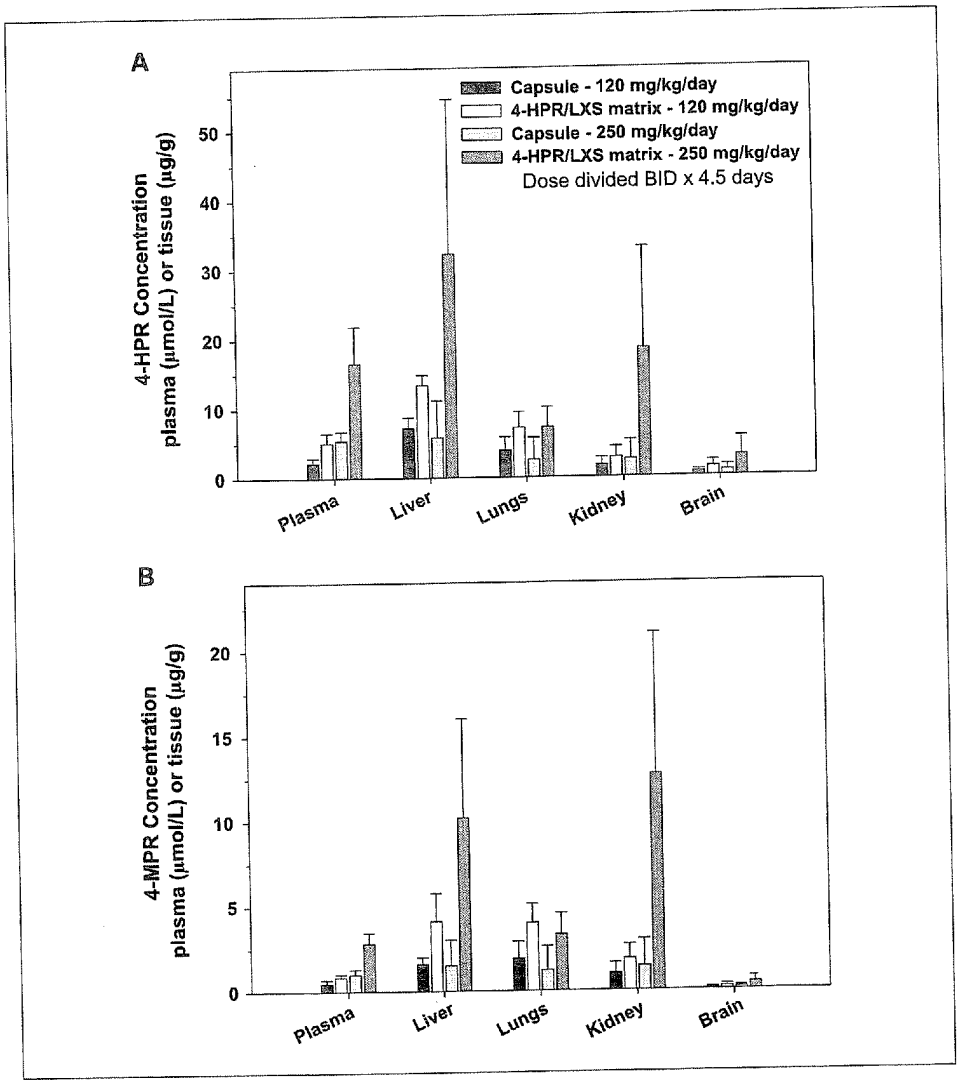


Fig. 1. Fenretinide (4-HPR) and metabolite (4-MPR) levels obtained in mouse plasma and tissues using fenretinide were administered in 4-HPR/LYM-X-SORB (*LXS*) organized lipid matrix or as the contents of NCI corn oil capsules. BALB/c mice were orally administered the extracted contents of NCI fenretinide capsules mixed in crushed mouse chow (4-HPR, 120 mg/kg/d, $n = 5$, *black columns*) or in a nutritional shake (4-HPR, 250 mg/kg/d, $n = 5$, *yellow columns*) or administered fenretinide inserted into LYM-X-SORB matrix (4-HPR/LYM-X-SORB molar ratio: 0.8:1.0; LYM-X-SORB composition, 1:3:3, molar ratio of lysophosphatidylcholine, monoglyceride, and free fatty acid). 4-HPR/LYM-X-SORB matrix was softened for delivery in water ($n = 5$), or in nutritional shake ($n = 5$), at two different doses (4-HPR, 120 mg/kg/d, *white columns*; 4-HPR, 250 mg/kg/d, *red columns*). Animals were administered drug in two equal daily doses (twice daily), for nine total doses, and sacrificed for analysis 3 h after the last dose. *A*, fenretinide (4-HPR) levels in plasma (μmol/L) and tissues (μg/g) were increased by delivery in LYM-X-SORB matrix compared with capsule contents: in plasma, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.01$); in liver, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.01$); in lungs, 120 mg/kg/d ($P < 0.02$) and 250 mg/kg/d ($P = 0.02$); in kidney, 120 mg/kg/d ($P = 0.14$) and 250 mg/kg/d ($P < 0.01$); and in brain, 120 mg/kg/d ($P = 0.05$) and 250 mg/kg/d ($P = 0.03$). *B*, metabolite (4-MPR) levels in plasma (μmol/L) and tissues (μg/g) were increased by fenretinide delivery in LYM-X-SORB matrix compared with NCI capsule contents: in plasma, 120 mg/kg/d ($P = 0.05$) and 250 mg/kg/d ($P < 0.01$); in liver, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.01$); in lungs, 120 mg/kg/d ($P < 0.01$) and 250 mg/kg/d ($P < 0.03$); in kidney, 120 mg/kg/d ($P < 0.04$) and 250 mg/kg/d ($P < 0.01$); and in brain, 120 mg/kg/d ($P = 0.04$) and 250 mg/kg/d ($P = 0.02$). Columns, 4-HPR concentration (*A*) and 4-MPR concentration (*B*); bars, SD.

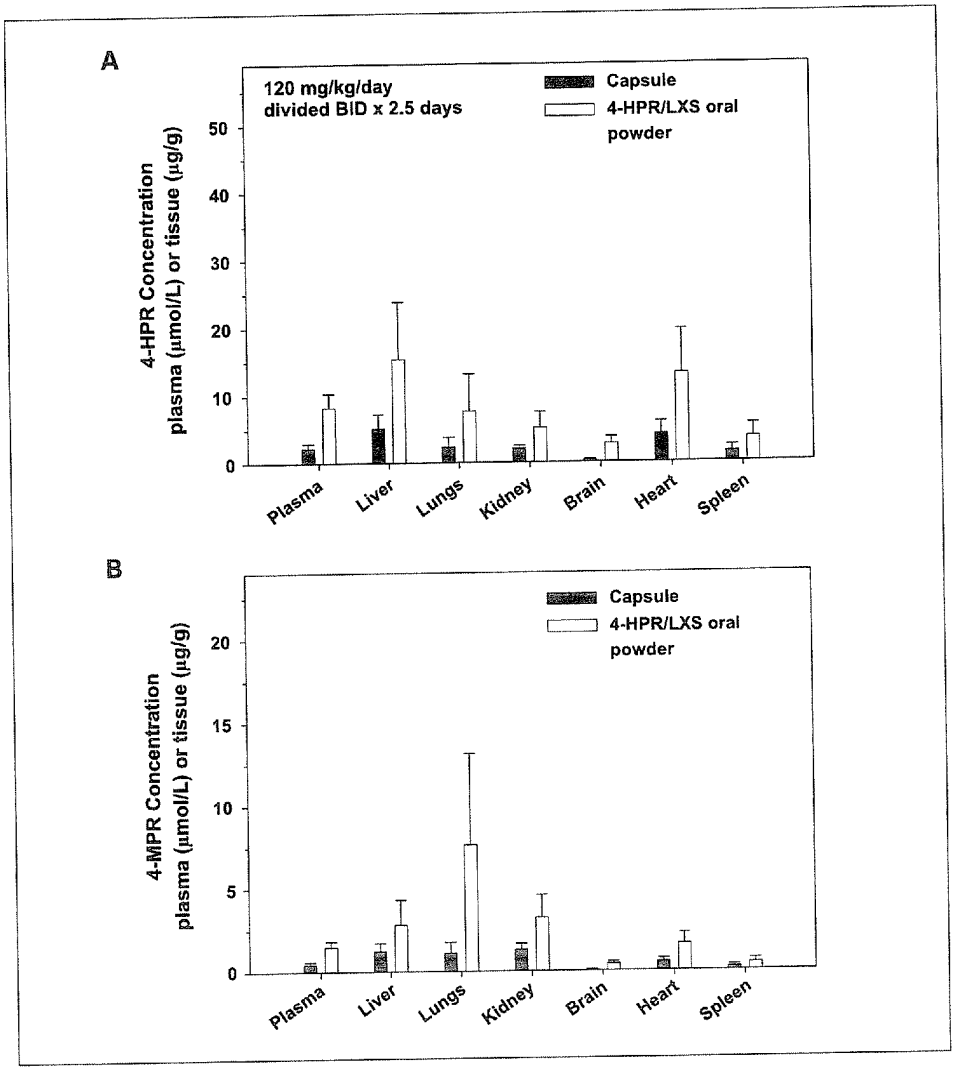


Fig. 2. Fenretinide (4-HPR) and metabolite (4-MPR) levels obtained in mouse plasma and tissues using 4-HPR administered in powderized 4-HPR/LYM-X-SORB matrix or as the contents of NCI corn oil capsules. Nude mice were orally administered fenretinide (4-HPR, 120 mg/kg/d) as the extracted contents of NCI fenretinide capsules mixed in a nutritional shake ($n = 9$, *black columns*) or in 4-HPR/LYM-X-SORB matrix (4-HPR/LYM-X-SORB molar ratio: 0.8:1.0; LYM-X-SORB composition, 1:4:2, molar ratio of lysophosphatidylcholine, monoglyceride, and free fatty acids) that was powderized with flour and sugar. 4-HPR/LYM-X-SORB oral powder (*white columns*) was slurried for delivery in water ($n = 5$) or in a nutritional shake ($n = 5$). Animals were administered drug in two equal daily doses (twice daily), for five total doses, and then sacrificed for analysis 4 h after the last dose. All animals were assayed for plasma levels. For tissue levels, animals administered 4-HPR/LYM-X-SORB oral powder in nutritional shake ($n = 5$) were analyzed for comparison against animals administered NCI capsule contents in nutritional shake ($n = 4$). *A*, fenretinide (4-HPR) levels in plasma and most tissues were increased by delivery in 4-HPR/LYM-X-SORB oral powder compared with capsule contents: in plasma, liver, kidney, brain, and heart, all $P < 0.05$; in lungs, $P = 0.09$; and in spleen, $P = 0.08$. *B*, metabolite (4-MPR) levels in plasma and most tissues were increased by 4-HPR delivery in powderized LYM-X-SORB matrix compared with NCI capsule contents: in plasma, kidney, brain, heart, and spleen, $P < 0.05$; in liver, $P < 0.08$; and in lungs, $P < 0.06$. Columns, 4-HPR concentration (*A*) and 4-MPR concentration (*B*); bars, SD.

Materials and Methods

Chemicals. 4-HPR (fenretinide), *N*-(4-methoxyphenyl)retinamide (4-MPR), and *N*-(4-ethoxyphenyl)retinamide were obtained from the NIH NCI. All chemicals were high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich Co. Matrigel Matrix HC was from BD Biosciences.

Cell lines. The human neuroblastoma cell lines, SMS-KCNR, CHLA-140, and CHLA-90, have been described previously (32–35). SMS-KCNR is a p53-functional cell line established at progressive disease after dual-agent induction chemotherapy; CHLA-140 is a multidrug-resistant, p53 functional cell line established at progressive disease after intensive multiagent chemotherapy that overexpresses MDR1; and CHLA-90 is a multidrug-resistant, p53 mutant cell line derived at relapse after myeloablative therapy and autologous bone marrow transplant that overexpresses MDR1.[6] Cell lines were maintained at 37°C in a humidified incubator containing 95% room air + 5% CO_2 atmosphere as described (3).

Drugs and formulation. Clinical grade, bulk fenretinide was obtained from the NCI and formulated into various compositions of LYM-X-SORB organized lipid matrix (4-HPR/LYM-X-SORB matrix) at a 4-HPR/LYM-X-SORB molar ratio of 0.8:1.0 under conditions of Good Manufacturing Practice by Avanti Polar Lipids, Inc. (36), operating under license from LYM-DRUG Products, LLC, and BioMolecular Products, Inc. (30). Fenretinide/LYM-X-SORB oral powder (4-HPR/LYM-X-SORB powder) was formulated by blending the 4-HPR/LYM-X-SORB matrix with sugar and wheat flour to final products that were either 2.2% or 3% by weight fenretinide, 16% or 22% LYM-X-SORB (lysophosphatidylcholine, monoglycerides, and free fatty acids, 1:4:2), ~20% sucrose, and the remainder wheat flour (37). 4-HPR capsules (100 mg in corn oil and polysorbate 80; NSC #374551, IND# 40294) were obtained from the NCI.

Animals. Five- to six-week-old BALB/c and Harlan athymic nude-Foxn1^{nu} mice were obtained from commercial vendors. For delivery of fenretinide from the (corn oil) capsules currently available from the NCI, the contents of the capsules were expressed after puncturing the capsule, or extracted with a needle and syringe, into an Eppendorf tube, and the amount of fenretinide obtained was quantified by HPLC assay. The capsule contents were then vortexed and mixed with crushed mouse chow or SlimFast liquid nutritional supplement ("nutritional shake") for oral or gavage delivery. For delivery of fenretinide in 4-HPR/LYM-X-SORB matrix (a hard wax at room temperature), 4-HPR/LYM-X-SORB matrix was compounded in crushed chow, or slurried in nutritional shake for oral syringe or gavage feeding, depending on the dose and volume required. For delivery of fenretinide in 4-HPR/LYM-X-SORB

[6] N. Keshelava, personal communication.

oral powder, the powder was mixed with water or nutritional shake for gavage feeding.

Cohorts of mice were administered the indicated dose of 4-HPR, on a divided daily schedule, for the number of doses indicated. In general, cohorts of five mice were used for each experimental condition or associated controls; the exact number of animals used for each experiment is listed in the figure legends. 4-HPR was well tolerated in all dose forms. The ability of mice to tolerate drug administration was assessed by daily examination of general activity and overall appearance and body weights at least twice weekly. Animals were sacrificed at 3 or 4 h after the last dose by carbon dioxide narcosis, and blood and organs were harvested and stored at -80°C for HPLC analysis. Fenretinide-containing materials were wrapped in foil, or kept in tinted tubes, to reduce exposure to light. For xenograft models, human neuroblastoma cell lines were established s.c. in nude mice. For passage and expansion, 16 to 20 million cells of established tumors were mixed in Matrigel Matrix HC per manufacturer's directions and injected s.c. (total volume of 0.2 mL) above the shoulder blade. All experiments were done using xenografts of ≤ passage 3 in mice. Drug treatment was begun when tumors measured 100 to 200 mm^3. Animals received 4-HPR/LYM-X-SORB oral powder, or powderized LYM-X-SORB matrix-alone (controls), mixed in 0.2 cc nutritional shake by gavage, in divided daily doses, Mondays to Fridays, for up to 18 weeks. Dosing by gavage was used to insure consistent dosing of the animals. Tumors were measured using calipers twice weekly and tumor volumes were calculated as 0.5 × height × width × length (38). Animals were sacrificed by carbon dioxide narcosis when tumor volumes exceeded 1,500 mm^3 per Institutional Animal Care and Use Committee guidelines. Treatment was well tolerated. All animals were housed and treated according to protocols approved by the Institutional Animal Care and Use Committee.

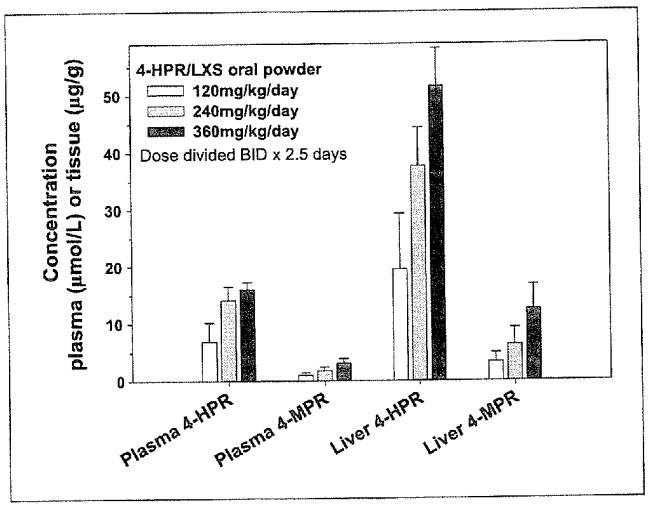


Fig. 3. Fenretinide (4-HPR) and metabolite (4-MPR) levels obtained in mouse plasma and liver using 2.2% and 3% clinical grade 4-HPR/LYM-X-SORB oral powder. Nude mice were gavaged with 4-HPR/LYM-X-SORB oral powder formulated under Good Manufacturing Practice conditions. 4-HPR/LYM-X-SORB matrix (4-HPR/LYM-X-SORB molar ratio: 0.8:1.0; LYM-X-SORB composition, 1:4:2, molar ratio of lysophosphatidylcholine, monoglyceride, and free fatty acid) was powderized with flour and sugar to final 4-HPR concentrations of 2.2% or 3%. Mice received the following: 4-HPR/LYM-X-SORB oral powder (4-HPR, 120 mg/kg/d, *white columns*) slurried for delivery in water (2.2% powder, $n = 5$; 3% powder, $n = 5$) or in a nutritional shake (3% powder, $n = 5$); 4-HPR/LYM-X-SORB oral powder (4-HPR, 240 mg/kg/d, *gray columns*) slurried for delivery in water (2.2% powder, $n = 5$; 3% powder, $n = 3$) or nutritional shake (3% powder, $n = 5$); or 4-HPR/LYM-X-SORB oral powder (4-HPR, 360 mg/kg/d, *black columns*) slurried for delivery in nutritional shake (3% powder, $n = 5$). Animals were administered drug in two equal daily doses (twice daily), for five total doses, and then sacrificed for analysis 4 h after the last dose. Both 4-HPR and 4-MPR levels in plasma and liver significantly increased with drug dosage: 120 versus 240 mg/kg/d, $P < 0.01$; 240 versus 360 mg/kg/d, $P < 0.05$. For a given fenretinide dosage level, there was no statistical differences in 4-HPR or 4-MPR plasma or liver levels obtained between animals receiving the 2.2% versus 3% powder or in plasma levels obtained using powder slurried in water versus nutritional shake. 4-HPR and 4-MPR liver levels trended lower in animals receiving oral powder in nutritional shake compared with water at 120 mg/kg/d ($P < 0.04$ and $P < 0.01$, respectively) but not at 240 mg/kg/d ($P = 0.20$ and $P = 0.14$, respectively). Columns, concentration; bars, SD.

Fenretinide assay. Fenretinide (4-HPR), and its principal metabolite, 4-MPR, were quantified in plasma and tissues using a HPLC method as described previously (39). Briefly, the plasma samples were added to internal standard, and ice-cold acetonitrile was added before homogenate was mixed by vortex and placed in an ultrasonic bath for 10 min. The mixture was then centrifuged at 10,000 × *g* at 4°C for 5 min. The supernatant was transferred to an autosampler and injected directly into the HPLC system. Tissues were homogenized using a Tissue Tearor (Biospec, Inc.) with a 1:3 ratio of physiologic saline (0.9% NaCl, w/v).

The HPLC used was a Waters Alliance 2690 Separation Module with a Waters 717 autosampler. Waters 2487 UV-Visible absorbance detector was set at a wavelength of 340 nm and autosampler temperature was set at 4°C. The column used for separation was a chemically bonded reversed-phase Symmetry C_{18} column (3.5 μm, 150 × 4.6 mm) with a Symmetry C_{18} guard column (5 μm, 3.9 × 20 mm). Isocratic elution with acetonitrile/water/glacial acetic acid (88:10:2, v/v/v) was used at a flow rate of 1.0 mL/min. Drug levels were calculated using the Empower Pro chromatographic data system. Calibration curves were prepared in methanol/acetonitrile (1:1, v/v) with concentrations ranging from 0.16 to 20 μg/mL depending on expected levels of analyte in the unknown samples. 4-HPR and 4-MPR were used as standards, and *N*-(4-ethoxyphenyl)retinamide was used as an internal standard. Blanks and low, middle, and high quality control samples were incorporated in each run. The same extraction procedure used for plasma and tissue samples was used for the calibration curves. The plasma concentration of 4-HPR and 4-MPR was presented in micrograms of drug per milliliter of plasma (μg/mL) or in micromolar per liter (μmol/L). The final tissue concentrations of drugs were presented in micrograms of drug per gram of wet tissue weight (μg/g).

Statistical analysis. The statistical significance of differences in means was evaluated by the Student's *t* test using Microsoft Excel 2000 software. *P* values were two sided. Analysis of human neuroblastoma murine xenograft models was by Kaplan-Meier log-rank analysis of survival. All tests were considered significant at $P < 0.05$.

Results

LYM-X-SORB matrix increased fenretinide levels. LYM-X-SORB organized lipid matrix can be formulated at various ratios of lysophosphatidylcholine, monoglycerides, and free fatty acids, to optimize incorporation of the target drug. Fenretinide (4-HPR) was incorporated into several hard, wax-like LYM-X-SORB lipid matrix (4-HPR/LYM-X-SORB matrix) preparations with the LYM-X-SORB matrix variously formulated with lysophosphatidylcholine/monoglycerides/free fatty acids ratios of 1:2:4; 1:4:2; or 1:3:3. Mice were orally dosed, either with these 4-HPR/LYM-X-SORB matrix formulations or with the expressed (expelled) contents of the currently available NCI fenretinide capsule (essentially a microcrystal slurry in corn oil). Levels of fenretinide, and its principal metabolite, 4-MPR, were then measured in plasma and tissues.

Figure 1 shows the fenretinide plasma and tissue levels obtained when 4-HPR incorporated into LYM-X-SORB matrix (1:3:3) was administered in various vehicles at doses of 120 and 250 mg 4-HPR/kg/d for 4.5 days compared with 4-HPR levels obtained by the contents of NCI 4-HPR capsules.

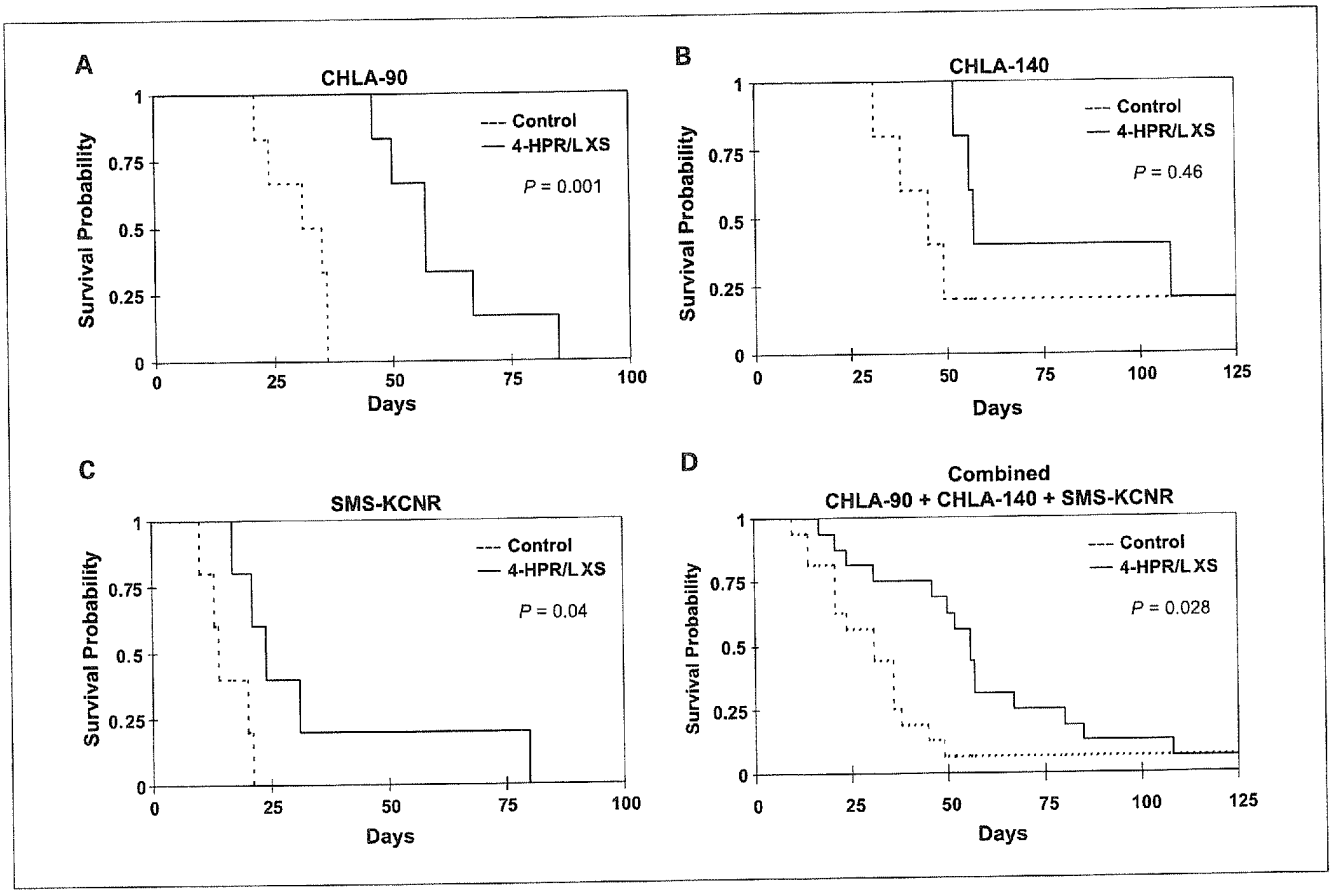


Fig. 4. 4-HPR/LYM-X-SORB oral powder prolonged survival in human neuroblastoma murine xenografts. Human neuroblastoma cell lines SMS-KCNR, CHLA-90, and CHLA-140 were established as s.c. xenografts in *nu/nu* mice. Animals received 120 mg/kg/d, by gavage, in daily divided doses, 5 d a week (CHLA-140, $n = 5$ per cohort), or 240 mg/kg/d, by gavage, in daily divided doses, 5 d a week (SMS-KCNR, $n = 5$ per cohort; CHLA-90, $n = 6$ per cohort). Control animals received powderized LYM-X-SORB matrix-alone. Animals were sacrificed when tumors reached 1,500 mm^3. *A*, CHLA-90. *B*, CHLA-140. *C*, SMS-KCNR. *D*, combined survival for SMS-KCNR, CHLA-90, and CHLA-140 ($n = 16$ per cohort). 4-HPR/LYM-X-SORB oral powder significantly prolonged survival in CHLA-90 and SMS-KCNR and in the combined survival data of the three models. Survival analysis by Kaplan-Meier log-rank test.

Compared with NCI capsule contents, at doses of 120 mg/kg/d, use of the wax-like 4-HPR/LYM-X-SORB matrix approximately doubled plasma and tissue levels of 4-HPR; at doses of 250 mg kg/d, 4-HPR plasma levels increased over 3-fold and tissue levels up to 6-fold (Fig. 1A). In accordance with results reported for rats (40), 4-HPR levels in the intact brain were the lowest of the tissues assayed, but even in brain, the 4-HPR/LYM-X-SORB matrix increased 4-HPR levels ~4-fold at doses of 250 mg/kg/d. 4-MPR levels in plasma and tissues generally mirrored 4-HPR levels, albeit at lower levels (Fig. 1B). Results obtained with 4-HPR/LYM-X-SORB matrix formulated at lysophosphatidylcholine/monoglycerides/free fatty acids ratios of 1:2:4 and 1:4:2 at doses of 120 mg/kg/d, for 4.5 days, were comparable with those obtained with the 1:3:3 matrix (data not shown).

As the three different LYM-X-SORB matrix lysophosphatidylcholine/monoglycerides/free fatty acids ratios tested seemed to be approximately equivalent in increasing 4-HPR bioavailability, formulation development proceeded with a LYM-X-SORB matrix ratio of 1:4:2, as LYM-X-SORB matrix-alone at this ratio had previously received phase I clinical testing as a nutritional supplement in cystic fibrosis patients (31).

Powderized LYM-X-SORB matrix increased fenretinide levels. LYM-X-SORB matrix, a hard wax at room temperature, can be consumed neat, or delivered in capsules. However, to maximize patient acceptance of the 4-HPR/LYM-X-SORB matrix, especially to children, and to facilitate delivery through nasogastric feeding tubes, the 4-HPR/LYM-X-SORB matrix (1:4:2) was "powderized" by blending the matrix oil with table sugar and wheat flour to a final 4-HPR concentration of 3% by weight. This formulation, 4-HPR/LYM-X-SORB oral powder, could be delivered neat, mixed with applesauce, or slurried in liquid carriers for delivery. 4-HPR/LYM-X-SORB oral powder delivered at 120 mg 4-HPR/kg/d, for 2.5 days, increased plasma levels ~3.5-fold and tissue levels up to 3-fold compared with capsule contents (Fig. 2A). 4-MPR levels generally mirrored 4-HPR levels, albeit at lower levels (Fig. 2B). 4-HPR/LYM-X-SORB oral powder at this dose increased 4-HPR plasma levels ($P < 0.01$) compared with the wax-like 4-HPR/LYM-X-SORB matrix delivered by itself (Fig. 1A), perhaps as a result of powderization facilitating absorption by reducing the particle size of the ingested LYM-X-SORB matrix.

Supported by the above data, a grant was secured from the NCI Developmental Therapeutics Program's Rapid Access to

Intervention Development program for the Good Manufacturing Practice (i.e., clinical grade) production of 4-HPR/LYM-X-SORB oral powder to support phase I trials in pediatrics and adults. 4-HPR/LYM-X-SORB oral powder was prepared at final 4-HPR concentrations of 2.2% and 3%. Both preparations were free-flowing powders; whereas the 2.2% powder had superior dissolution properties in liquids, the 3% powder could be adequately slurried in liquids and had a consistency more amenable to direct ingestion or mixing with solids for ingestion. Clinical grade 4-HPR/LYM-X-SORB oral powders (2.2% and 3%) were administered to mice at doses from 120 to 360 mg/kg/d, for 2.5 days, and plasma and liver 4-HPR and 4-MPR levels were assayed (Fig. 3). 4-HPR levels in plasma and liver were increased 3- to 7-fold compared with 4-HPR delivered at similar doses using capsule contents (Figs. 1 and 2) and the levels obtained were dose responsive in this range.

Fenretinide/LYM-X-SORB oral powder prolonged survival in neuroblastoma xenograft models. Three human neuroblastoma cell lines were established as xenografts in nude mice. SMS-KCNR is drug-sensitive *in vitro*, whereas CHLA-140 and CHLA-90 are multidrug-resistant *in vitro* (32–35). CHLA-90 has loss of p53 function via a *TP53* mutation; both CHLA-140 and CHLA-90 overexpress the *MDR1* gene.[6] Xenografts received 4-HPR/LYM-X-SORB oral powder, or powderized LYM-X-SORB matrix-alone (controls), by gavage for up to 18 weeks. Treatment with 4-HPR/LYM-X-SORB oral powder prolonged survival in two of the three xenograft models and for the combined data from all three models, as shown by Kaplan-Meier analysis (Fig. 4). This showed that fenretinide delivered in 4-HPR/LYM-X-SORB oral powder was both bioavailable and bioactive. Chronic dosing of 4-HPR/LYM-X-SORB oral powder seemed well tolerated by mice; mouse body weights were not different from the controls (Fig. 5).

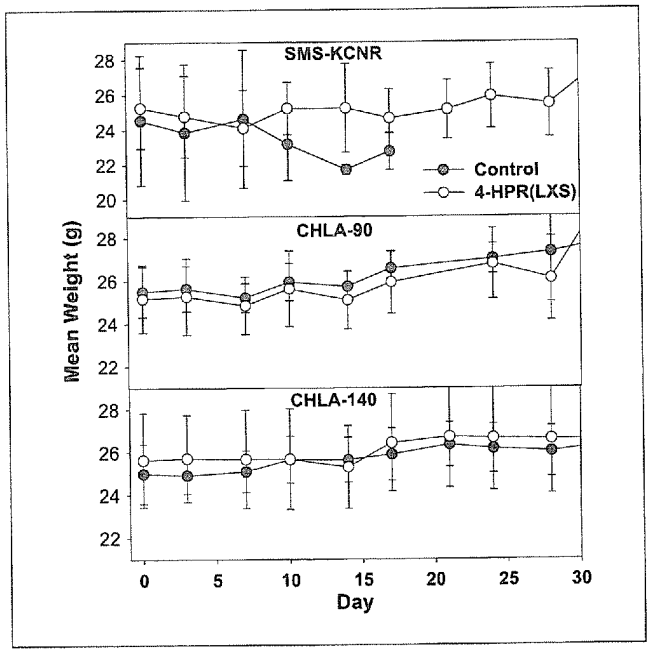


Fig. 5. Body weights of mice during the initial 30 d of the experiment presented in Fig. 4. Weight comparisons could only be carried out for the initial 30 d (a total of four 5-d courses of 4-HPR/LYM-X-SORB oral powder) as mice were culled due to tumor burdens. Statistical differences were assessed at day 14; there was no significant difference ($P > 0.05$) in body weights for those mice treated with 4-HPR/LYM-X-SORB compared with controls for mice carrying CHLA-90 and CHLA-140 xenografts. The SMS-KCNR control mice had significantly ($P = 0.035$) lower weights than 4-HPR-treated mice due to morbidity from tumor xenograft growth.

Discussion

To date, clinical investigations of 4-HPR (fenretinide) have principally focused on its oncologic use as a chemopreventive or chemotherapeutic agent, although recent preclinical data suggest that fenretinide may have clinical potential in the prevention or treatment of obesity-related type II diabetes (41, 42) and/or retinal degenerative diseases, such as age-related macular degeneration and Stargardt's macular degeneration (43–45). Direct clinical activity observed with fenretinide in cancers to date has been modest, possibly due to the low drug levels obtained, although tumor responses and/or disease stability have been noted in neuroblastoma (27, 46), platinum-refractory ovarian cancer (47), and oral leukoplakia (48). Fenretinide has been generally well tolerated on both low-dose and high-dose schedules with a reversible decrease in night vision being the most common side effect (25). Decreased night vision (nyctalopia), however, has not prevented daily fenretinide administration for up to 5 years (49).

One impediment to the clinical development of fenretinide has been its historical delivery vehicle, a large, corn oil-based capsule of limited bioavailability that delivers plasma levels with a wide interpatient variability. In one trial, the daily consumption of up to 4,000 mg/m^2 fenretinide (40 capsules/m^2), for 4 weeks of every 5 weeks, resulted in mean plasma levels of only 12.9 μmol/L (28). Patient dissatisfaction with the capsule formulation on high-dose schedules has been noted (28, 50).

In an attempt to improve drug delivery and maximize tumor response, we have placed fenretinide in a novel, organized lipid matrix, called LYM-X-SORB, and powderized the 4-HPR/LYM-X-SORB matrix for delivery. As the data presented show, this formulation increased plasma and tissue levels in mice from 3- to 7-fold compared with identical doses delivered as the contents of fenretinide in corn oil capsules and prolonged survival in human neuroblastoma xenograft models. We observed that 4-HPR delivered in any form of LYM-X-SORB matrix increased 4-HPR bioavailability. However, working with the hard, wax-like LYM-X-SORB matrix produced intermouse variability in drug levels obtained, likely due to the technical difficulties associated with its consistency. Powderizing the 4-HPR/LYM-X-SORB matrix produced a product that was easier to deliver mechanically and that obtained higher drug levels with a lower interanimal variance.

In the formulation ultimately developed for clinical trials, fenretinide/LYM-X-SORB oral powder has roughly the consistency of brown sugar. It is intended to be dissolved or slurried in nonmilk fat–containing liquids, mixed with foods, or consumed neat for patient delivery. It is hoped that this powderized format will be user-friendly to patients and facilitate compliance on high-dose fenretinide schedules.

Indeed, fenretinide/LYM-X-SORB oral powder slurried into a liquid nutritional supplement has been delivered via nasogastric tube to a cancer patient with restricted oral intake.[7] Powderized LYM-X-SORB organized lipid matrix (as described in this report, but lacking fenretinide) will also be tested as a dietary supplement in cystic fibrosis patients in an upcoming randomized, double-blinded, nutritional intervention study funded by the NIH National Institutes of Diabetes, Digestive and Kidney Diseases (grant 2 R44 DK060302-02A1).

Based on the preclinical data reported here, a phase I trial of fenretinide/LYM-X-SORB oral powder (NL2004-04, B. Maurer, Chair) in pediatric neuroblastoma patients is in progress in the NCI-funded, New Approaches to Neuroblastoma Therapy consortium[8] with the drug supplied via by a Rapid Access to Intervention Development grant from the NCI Developmental Therapeutics Program. It is anticipated that this novel formulation will deliver higher and/or more uniform fenretinide levels for use in future single- and combination-agent clinical trials in cancer and other disease states.

[7] B.J. Maurer, unpublished data.

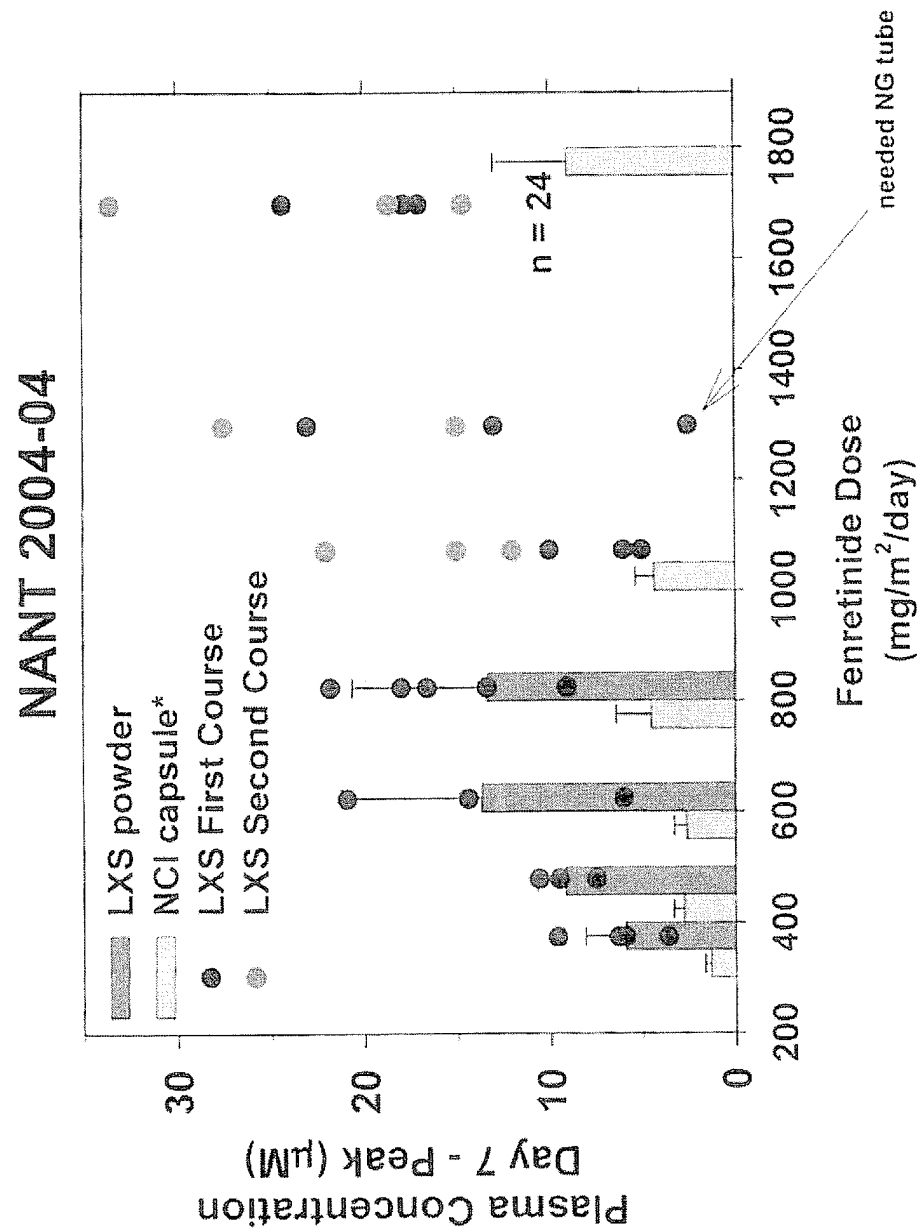
[8] http://www.nant.org

References

1. Ponzoni M, Bocca P, Chiesa V, et al. Differential effects of *N*-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: apoptosis versus differentiation. Cancer Res 1995;55:853–61.
2. Di Vinci A, Geido E, Infusini E, Giaretti W. Neuroblastoma cell apoptosis induced by the synthetic retinoid *N*-(4-hydroxyphenyl)retinamide. Int J Cancer 1994;59:422–6.
3. Maurer BJ, Melton L, Billups C, Cabot MC, Reynolds CP. Synergistic cytotoxicity in solid tumor cell lines between *N*-(4-hydroxyphenyl)retinamide and modulators of ceramide metabolism. J Natl Cancer Inst 2000;92:1897.
4. Ziv Y, Gupta MK, Milsom JW, Vladisavljevic A, Brand M, Fazio VW. The effect of tamoxifen and fenretinimide on human colorectal cancer cell lines *in vitro*. Anticancer Res 1994;14:2005–9.
5. Hsieh TC, Ng C, Wu JM. The synthetic retinoid *N*-(4-hydroxyphenyl) retinamide (4-HPR) exerts antiproliferative and apoptosis-inducing effects in the androgen-independent human prostatic JCA-1 cells. Biochem Mol Biol Int 1995;37:499–506.
6. Igawa M, Tanabe T, Chodak GW, Rukstalis DB. *N*-(4-hydroxyphenyl) retinamide induces cell cycle specific growth inhibition in PC3 cells. Prostate 1994;24:299–305.
7. Kazmi SM, Plante RK, Visconti V, Lau CY. Comparison of *N*-(4-hydroxyphenyl)retinamide and all-*trans*-retinoic acid in the regulation of retinoid receptor-mediated gene expression in human breast cancer cell lines. Cancer Res 1996;56:1056–62.
8. Coradini D, Biffi A, Pellizzaro C, Pirronello E, Di Fronzo G. Combined effect of tamoxifen or interferon-β and 4-hydroxyphenylretinamide on the growth of breast cancer cell lines. Tumor Biol 1997;18:22–9.
9. Supino R, Crosti M, Clerici M, et al. Induction of apoptosis by fenretinide (4HPR) in human ovarian carcinoma cells and its association with retinoic acid receptor expression. Int J Cancer 1996;65:491–7.
10. Sabichi AL, Hendricks DT, Bober MA, Birrer MJ. Retinoic acid receptor β expression and growth inhibition of gynecologic cancer cells by the synthetic retinoid *N*-(4-hydroxyphenyl) retinamide. J Natl Cancer Inst 1998;90:597–605.
11. Formelli F, Cleris L. Synthetic retinoid fenretinide is effective against a human ovarian carcinoma xenograft and potentiates cisplatin activity. Cancer Res 1993;53:5374–6.
12. Kalemkerian GP, Slusher R, Ramalingam S, Gadgeel S, Mabry M. Growth inhibition and induction of apoptosis by fenretinide in small-cell lung cancer cell lines. J Natl Cancer Inst 1995;87:1674–80.
13. Delia D, Aiello A, Lombardi L, et al. *N*-(4-hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. Cancer Res 1993;53:6036–41.
14. Chan LN, Zhang S, Shao J, Waikel R, Thompson EA, Chan TS. *N*-(4-hydroxyphenyl)retinamide induces apoptosis in T lymphoma and T lymphoblastoid leukemia cells. Leuk Lymphoma 1997;25:271–80.
15. O'Donnell PH, Guo WX, Reynolds CP, Maurer BJ. *N*-(4-hydroxyphenyl)retinamide increases ceramide and is cytotoxic to acute lymphoblastic leukemia cell lines, but not to non-malignant lymphocytes. Leukemia 2002;16:902–10.
16. Wu JM, Dipietrantonio AM, Hsieh TC. Mechanism of fenretinide (4-HPR)-induced cell death. Apoptosis 2001;6:377–88.
17. Delia D, Aiello A, Formelli F, et al. Regulation of apoptosis induced by the retinoid *N*-(4-hydroxyphenyl) retinamide and effect of deregulated bcl-2. Blood 1995;85:359–67.
18. Zou C-P, Kurie JM, Lotan D, Zou C-C, Hong WK, Lotan R. Higher potency of *N*-(4-hydroxyphenyl)retinamide than all-*trans*-retinoic acid in induction of apoptosis in non-small cell lung cancer cell lines. Clin Cancer Res 1998;4:1345–55.
19. Maurer BJ, Metelitsa LS, Seeger RC, Cabot MC, Reynolds CP. Increase of ceramide and induction of mixed apoptosis/necrosis by *N*-(4-hydroxyphenyl)-retinamide in neuroblastoma cell lines. J Natl Cancer Inst 1999;91:1138–46.
20. Oridate N, Suzuki S, Higuchi M, Mitchell MF, Hong WK, Lotan R. Involvement of reactive oxygen species in *N*-(4-hydroxyphenyl)retinamide-induced apoptosis in cervical carcinoma cells. J Natl Cancer Inst 1997;89:1191–8.
21. Delia D, Aiello A, Meroni L, Nicolini M, Reed JC, Pierotti MA. Role of antioxidants and intracellular free radicals in retinamide-induced cell death. Carcinogenesis 1997;18:943–8.
22. Dipietrantonio A, Hsieh T-C, Olson SC, Wu JM. Regulation of G_1/S transition and induction of apoptosis in HL-60 leukemia cells by fenretinide (4HPR). Int J Cancer 1998;78:53–61.
23. Batra S, Reynolds CP, Maurer BJ. Fenretinide cytotoxicity for Ewing's sarcoma (ES) and primitive neuroectodermal tumor (PNET) cell lines is decreased by hypoxia and synergistically enhanced by ceramide modulators. Cancer Res 2004;64:5415–24.
24. Rehman F, Shanmugasundaram P, Schrey MP. Fenretinide stimulates redox-sensitive ceramide production in breast cancer cells: potential role in drug-induced cytotoxicity. Br J Cancer 2004;91:1821–8.
25. Torrisi R, Parodi S, Fontana V, et al. Factors affecting plasma retinol decline during long-term administration of the synthetic retinoid fenretinide in breast cancer patients. Cancer Epidemiol Biomarkers Prev 1994;3:507–10.
26. Jasti BR, LoRusso PM, Parchment RE, et al. Phase I clinical trial of fenretinide (NSC374551) in advanced solid tumors. Proc Am Soc Clin Oncol 2001;20:122a.
27. Villablanca JG, Krailo MD, Ames MM, Reid JM, Reaman GH, Reynolds CP. Phase I trial of oral fenretinide in children with high risk solid tumors: a report from the Children's Oncology Group (CCG 09709). J Clin Oncol 2006;24:3423–30.
28. Garaventa A, Luksch R, Piccolo MS, et al. Phase I trial and pharmacokinetics of fenretinide in children with neuroblastoma. Clin Cancer Res 2003;9:2032–9.
29. Yesair DW. Composition for delivery of orally administered drugs and other substances. United States patent U.S. 4874795. 1989 October 17.
30. Yesair DW. Composition for the delivery of orally administered drugs and other substances. United States patent U.S. 5972911. 1999 October 26.
31. Lepage G, Yesair DW, Ronco N, et al. Effect of an organized lipid matrix on lipid absorption and clinical outcomes in patients with cystic fibrosis. J Pediatr 2002;141:178–85.
32. Keshelava N, Tsao-Wei D, Reynolds CP. Pyrazoloacridine is active in multidrug-resistant neuroblastoma cell lines with nonfunctional p53. Clin Cancer Res 2003;9:3492–502.
33. Keshelava N, Zuo JJ, Chen P, et al. Loss of p53 function confers high-level multidrug resistance in neuroblastoma cell lines. Cancer Res 2001;61:6185–93.
34. Keshelava N, Seeger RC, Groshen S, Reynolds CP. Drug resistance patterns of human neuroblastoma cell lines derived from patients at different phases of therapy. Cancer Res 1998;58:5396–405.
35. Keshelava N, Groshen S, Reynolds CP. Cross-resistance of topoisomerase I and II inhibitors in neuroblastoma cell lines. Cancer Chemother Pharmacol 2000;45:1–8.
36. Yesair DW, Shaw WA, Burgess SW, McKee RT. Modification of solid 3-sn-phosphoglycerides. United States patent U.S. 2004/0259837 A1.
37. Maurer BJ, Reynolds CP, Yesair DW, McKee RT, Burgess SW, Shaw WA. Oral compositions of fenretinide having increased bioavailability and methods of using the same. United States patent U.S. 2005/0106216 A1.
38. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol 1989;24:148–54.
39. Vratilova J, Frgala T, Maurer BJ, Patrick CP. Liquid chromatography method for quantifying *N*-(4-hydroxyphenyl)retinamide and *N*-(4-methoxyphenyl)retinamide in tissues. J Chromatogr B Analyt Technol Biomed Life Sci 2004;808:125–30.
40. Le Doze F, Debruyne D, Albessard F, Barre L, Defer GL. Pharmacokinetics of all-*trans* retinoic acid, 13-cis retinoic acid, and fenretinide in plasma and brain of Rat. Drug Metab Dispos 2000;28:205–8.
41. Yang Q, Graham TE, Mody N, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356–62.

42. Graham TE, Yang Q, Bluher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006; 354:2552–63.
43. Bui TV, Han Y, Radu RA, Travis GH, Mata NL. Characterization of native retinal fluorophores involved in biosynthesis of A2E and lipofuscin-associated retinopathies. J Biol Chem 2006;281:18112–9.
44. Radu RA, Han Y, Bui TV, et al. Reductions in serum vitamin A arrest accumulation of toxic retinal fluorophores: a potential therapy for treatment of lipofuscin-based retinal diseases. Invest Ophthalmol Vis Sci 2005;46:4393–401.
45. Radu RA, Mata NL, Nusinowitz S, Liu X, Travis GH. Isotretinoin treatment inhibits lipofuscin accumulation in a mouse model of recessive Stargardt's macular degeneration. Novartis Found Symp 2004; 255:51–63.
46. Villablanca JG, London W, McGrady P, et al. Children's Oncology Group Protocol ANBL0321: a phase II study of 4-hydroxyphenylretinamide (4-HPR/fenretinide) in pediatric patients with refractory or recurrent neuroblastoma. Adv Neuroblast Res 2006;122.
47. Garcia AA, Morgan R, McNamara M, et al. Phase II trial of fenretinide (4-HPR) in recurrent ovarian and primary peritoneal carcinoma: a California Cancer Consortium Trial. Proc Am Soc Clin Oncol 2004;23:461.
48. Lippman SM, Lee JJ, Martin JW, et al. Fenretinide activity in retinoid-resistant oral leukoplakia. Clin Cancer Res 2006;12:3109–14.
49. Veronesi U, de Palo G, Marubini E, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. J Natl Cancer Inst 1999;91:1847–56.
50. Otterson GA, Lavelle J, Villalona-Calero MA, et al. A phase I clinical and pharmacokinetic study of fenretinide combined with paclitaxel and cisplatin for refractory solid tumors. Invest New Drugs 2005;23:555–62.

APPENDIX 8



*Villablanca, et al, J. Clin Oncology, 24:3423-3430, 2006.
Reynolds, et al, ASCO, 2007.

APPENDIX 9

From: Marachelian, Araz [AMarachelian@chla.usc.edu]
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To: Maurer, Barry
Subject: RE: sources of NANT clinical trials funding to list for ASCO

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Phase I study of fenretinide (4-HPR)/ Lym-X-Sorb™(LXS) oral powder in patients with recurrent or resistant neuroblastoma. New Approaches to Neuroblastoma Therapy (NANT) consortium trial.

Author Block A. Marachelian, M. H. Kang, K. Hwang, J. G. Villablanca, S. Groshen, K. K. Matthay, J. Maris, K. B. DeSantes, C. P. Reynolds, B. J. Maurer, New Approaches to Neuroblastoma Therapy; Childrens Hospital Los Angeles, Los Angeles, CA; Texas Tech University Health Sciences Center, Lubbock, TX; University of Southern California, Los Angeles, CA; Univerisity of California, San Francisco, San Francisco, CA; Childrens Hospital Philadelphia, Philadelphia, PA; University of Wisconsin, Madison, WI

Abstract:
Background: Fenretinide (4-HPR), a cytotoxic retinoid, achieved limited and variable plasma levels when tested in a corn oil-based capsule. 4HPR/LXS® oral powder is a new formulation intended to increase bioavailability, especially in children.
Methods: This trial sought to define the toxicities, dose limiting toxicities (DLTs), maximum tolerated dose (MTD), and pharmacokinetics of 4-HPR/LXS® oral powder when given mixed in Slim-Fast® nutritional shakes, twice a day for 7 days, every 21 days. Eligible patients had high-risk neuroblastoma with recurrent/progressive disease, or disease refractory or persistent after frontline therapy. Dose escalation was in 30% increments using the traditional 3 + 3 design. Plasma levels were measured by HPLC.
Results:32 patients accrued to 8 Dose Levels (DL) (352 - 2210 mg/m^2/day); 30 patients were evaluable. No MTD was identified. There was a DLT elevation of alkaline phosphatase on DL1. No other DLT's were observed. Other toxicities included dry skin, elevated triglycerides, reversible nyctalopia, and transient transaminase elevation. Course 1 Day 7 Peak 4HPR plasma levels (**Dose** (mg/m2/day) - mean, (range)) were: **352** - 6 µM (3.8-9.6); **458**- 11.5 µM (9.7-14.9); **595**-17.6 µM (6-24.3); **774**- 15 µM (9.1-25.3); **1006**- 6.7 µM (5.2-9.2); **1308**- 13.9 µM (2.9-23.8); **1700**- 19.7 µM (17.3-24.3); and **2210**- 10.8 µM (4-16.5). Course 2 Day 7 Peak plasma levels trended higher than Course 1 at DL5-8. Three patients with isolated bone marrow disease and one with MIBG avid bone lesions, had complete responses (DL4, DL4, DL7, DL8) receiving 10, 17, 18 and 10 courses of therapy, respectively. Six patients had stable disease for 4-27 courses (median 5.5) (DL3, DL4, DL4, DL5, DL6, DL8). 20 patients had progressive disease. Central review of responses is pending.
Conclusions:
4-HPR/LXS® oral powder was well tolerated, obtained 2 - 5 fold higher 4HPR plasma levels than fenretinide capsules on the same dose and schedule ($P < 0.01$), and showed anti-tumor activity (complete responses in 4/15 patients at DL4-8). Based on pharmacokinetic data, a recommended Phase II dose and schedule is 1700 mg/m^2/day x 7days every 3 weeks.

Author Disclosure Information: **A. Marachelian**, None; **M.H. Kang**, None; **K. Hwang**, None; **J.G. Villablanca**, None; **S. Groshen**, None; **K.K. Matthay**, None; **J. Maris**, None; **K.B. DeSantes**, None; **C.P. Reynolds**, None; **B.J. Maurer**, None.

Phase I Study of Fenretinide Lym-X-Sorb® (LXS) 3% Oral Powder in Patients with Recurrent or Resistant Neuroblastoma

New Approaches to Neuroblastoma Therapy (NANT) Consortium Trial

Barry J. Maurer, MD PhD
Study Chair and IND Sponsor

A. Marachelian, M. H. Kang, K. Hwang, J. G. Villablanca, S. Groshen, K. K. Matthay, J. Maris, K. B. DeSantes and C. P. Reynolds

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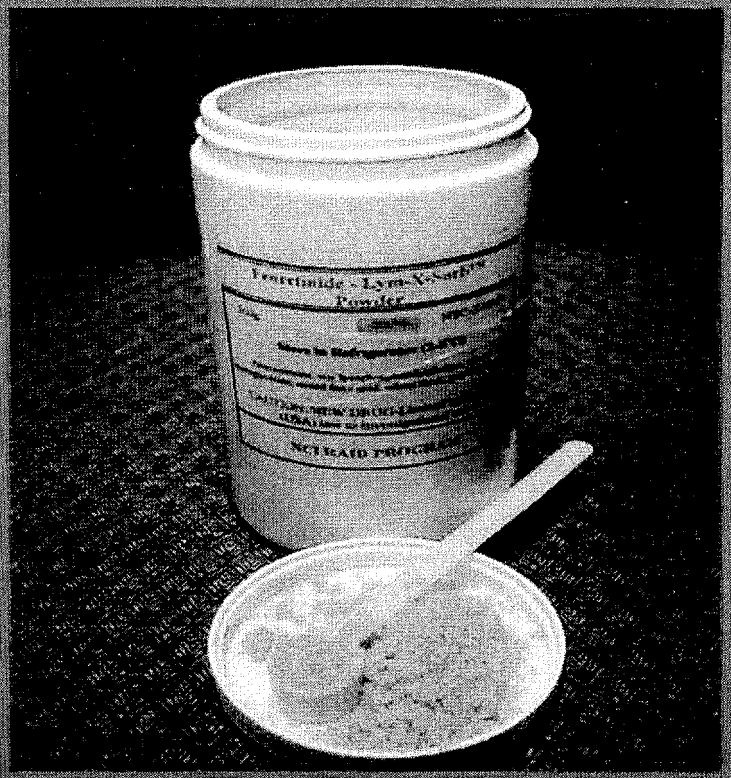
Study Schema

7 Day 4-HPR/LXS™ oral powder

21 Day treatment course



Scoops of fenretinide oral powder mixed in Slim-Fast® nutritional shakes.
Dosing done by standardized surface area chart

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Responses

- 4 complete responses (10-26 courses)
- 6 stable disease (4-27 courses)
- All complete responses on dose levels 4-8 (n=18)

Conclusions

- 4-HPR/LXS™ oral powder is well tolerated in a heavily pre-treated neuroblastoma patients.
- Only 3/32 patients were unable to ingest prescribed drug.
- Peak and steady state levels were 2-5 fold higher than with 4-HPR corn oil capsules at all dose levels tested to date.
- 4-HPR/LXS™ oral powder appears to have anti-tumor activity in neuroblastoma

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